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SOME FACTORS IN SECRETION BY SUBMAXILLARY GLANDS OF CATS

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The work to be reported here was undertaken to study the effects of various frequencies of stimulation of the chorda tympani upon the secretory response of the submaxillary gland, and to investigate some of the factors in the functioning of the gland.

Experimental. Cats anesthetised with Dial were used in all experiments. The submaxillary duct was cannulated with a capillary glass tube, and salivary flow was registered by a drop counter similar to that of Gesell (1929). The chorda tympani was dissected out in the neck and stimulated through platinum electrodes by a thyratron stimulator. Stimulation with pilocarpine was achieved either by intermittent injection into a femoral vein or by continuous injection into the isolated arterial supply of the submaxillary gland. Blood flows were obtained by isolating the return from the stimulated gland and registering the venous flow with another drop counter, the blood of the animal having been rendered non-coagulable by intravenous injection of "Liquoid" (Hoffman-LaRoche). Potassium was determined by the method used by Fenn et al. (1938).

The effects of various frequencies of stimulation of the chorda tympani upon the secretory response of the submaxillary gland are shown in figure 1. This figure presents the response of a single submaxillary gland during each minute of the first five minutes of stimulation at various frequencies but with constant strength of stimulus. The curves drawn here are typical of those obtained in five such experiments. These graphs show that the salivary secretion during the first minute of stimulation was practically constant for a fairly wide range of frequencies. However, at a frequency of 60 per sec. the initial secretion was small. The optimal rate of stimulation appeared to be about 9 per sec. At this frequency the

initial high secretory output was best maintained. Essentially the same conclusions can be reached from a consideration of figures 2 and 3, although there the picture is somewhat complicated by the fact that each curve represents a separate animal.

At first these findings seem to conflict with those of Rosenblueth (1932), who reported that the optimal frequency for stimulation of the chorda tympani was 36 shocks per sec. Rosenblueth's experiments lasted for

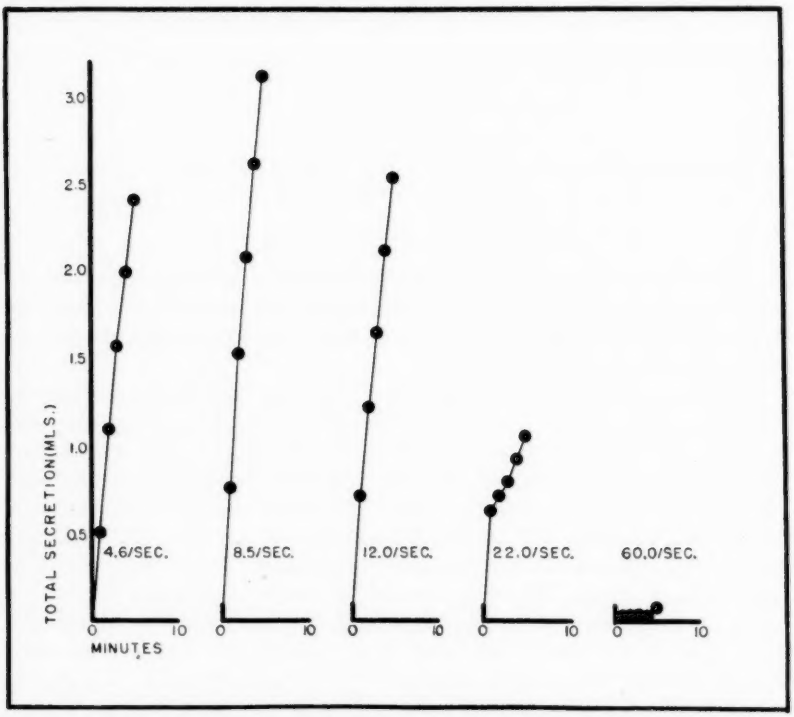

Fig. 1. Responses of a single submaxillary gland during each minute of the first five minutes of electrical stimulation of the chorda at various frequencies but with constant strength of stimulus.

only 30 seconds; there is evidence in his paper that if stimulation had been prolonged, a lower value for the optimal frequency would have been obtained. By reference to figure 7 of Rosenblueth's paper it can be seen that while frequencies of stimulation of around 25 per sec. produced greater initial rates of secretion than did those of around 9 per sec., the primary rate was better maintained at the lower frequency even for the half-minute duration of those experiments. Frequencies of stimulation

below about 9 per sec. produced well-maintained rates of secretion, but these rates were below those produced by the 9 per sec. frequency. Therefore it seems likely that there is no real disagreement between Rosenblueth's work and the present experiments.

Figure 2 presents the results of nine experiments in which electrical stimulation of the chorda tympani at various frequencies was continued for about three hours, the strength of stimulus in any one experiment being constant throughout. The fact that each curve represents a different

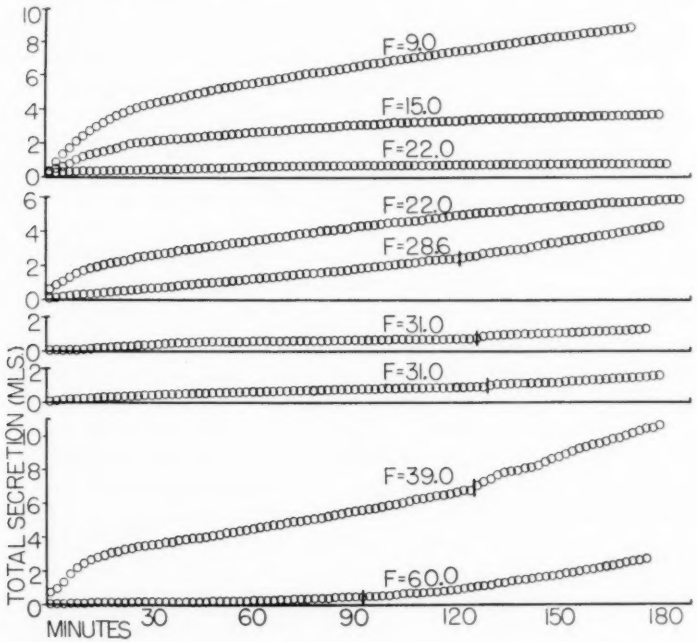

Fig. 2. Variations with time of the total amount of saliva produced by submaxillary glands stimulated for long periods at various frequencies.

animal probably explains in part the different amounts of total secretion obtained in the various experiments. However, the shape of the curves is of more interest at present than their magnitude.

It will be seen that some of the graphs of figure 2 have a vertical line drawn on them. This line indicates what seems to be a fairly sudden increase in the rate of secretion, occurring between the 123rd and 131st minute after commencement of stimulation at frequencies between 28.6 and 39.0 per sec. In the experiment with stimulation at 60.0 per sec. this sudden increased rate of salivation appeared at the 94th minute. The

experiments at frequencies below 28.6 shocks per sec. showed no sign of such an increase, even though one of the experiments at a stimulation rate of 22.0 per sec. was prolonged to the 218th minute after commencement of stimulation.

The sigmoidal first portions of the curves of figure 2 suggest that careful analysis of this section of these graphs would reveal a rapid, a slow and again a rapid rate of salivary production. To test this supposition, the rates of secretion during each of the first twenty minutes for some of the experiments have been plotted in figure 3. Here it appears that in every case there was an initial period of a relatively high rate of secretion, followed by a falling off. In the experiments at frequencies of 39.0 or less shocks per sec. there was a secondary increase in rate at times varying from the sixth to the twelfth minute of stimulation. During this period the rate of secretion might increase to as much as 40 per cent of the rate during the first minute. Then the speed of secretion decreased again and leveled off into the continuous and uniform output making up the major portion of the curves of figure 2.

These changes in rate of secretion show a certain similarity to the changes of tension of muscles during long-continued stimulation (Rosenblueth and Morison, 1937) (Rosenblueth and Luco, 1939). In particular, the late increase in rate of saliva production shows similarity to Rosenblueth's "fifth stage" in muscle by beginning earlier with higher frequencies of stimulation.

By referring again to figure 3 it will be seen that the experiment with stimulation at a frequency of 60 shocks per sec. had only fleetingly a relatively high rate of secretion, salivary production falling to a uniform low level within two minutes of commencement of stimulation. By following the interpretation of a similar situation in muscle by Rosenblueth and Luco (1939), it could be said that there was absence of stage two with merging of stages one and three. This latter combined phase was completed in the first minute or so, and stage four then began and lasted until the beginning of stage five at the 94th minute.

Another series of experiments was undertaken to study simultaneous variations in blood and salivary flows with both chorda and pilocarpine stimulations. The submaxillary glands of seven animals were stimulated by electrical excitation of the chorda tympani, at frequencies ranging from 7.9 to 31.0 shocks per sec.; there were five animals with pilocarpine as stimulant of the gland.

Table 1 presents the averaged data from these experiments together with other similar averages collected from the literature. It will be noted that the figures for resting blood flow in the cats are markedly lower than those quoted for dogs. This difference can be very largely removed by calculating the figures on the basis of the weight of the submaxillary gland.

When this is done, it is found that the cat had an average resting blood flow through its submaxillary gland of 0.20 ml. per min. per gram of tissue; from the data of Anochin, Goldberg and Samarina (1930) the dog had a resting blood flow of 0.25 ml. per min. per gram of tissue, and from the data of Tatibana (1936–38) it had one of 0.27 ml. per min. per gram of gland.

The figures of table 1 show that with secretion by the submaxillary gland there was an increased blood flow through the gland whatever the stimulant. However, they also seem to show that the amount of saliva produced with a given blood flow was greater with pilocarpine stimulation

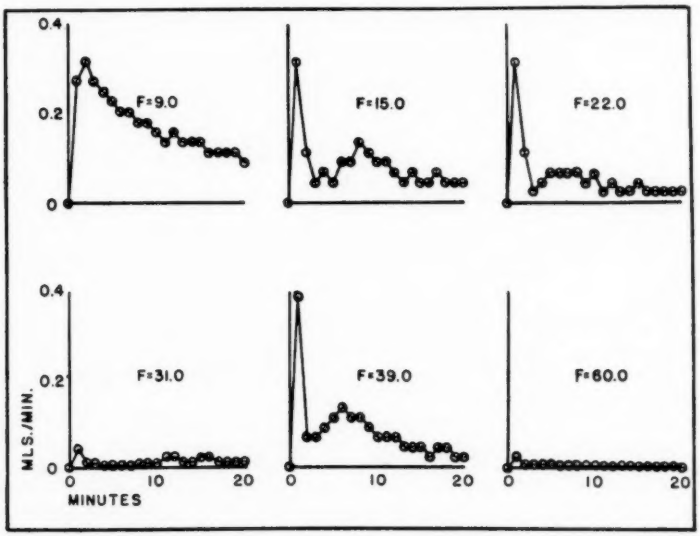

Fig. 3. Variations with time of the rate of saliva production by submaxillary glands stimulated at various frequencies.

than with chorda excitation. The latter point will be discussed further in a subsequent portion of this paper.

From the data of Barcroft, Barcroft and Kato and Tatibana in table 1, it can be calculated that the assumption that arterial blood flow equals venous blood flow plus saliva flow yields values for arterial flow low by only 2.3 per cent of the correct figure. Therefore, in the experiments reported here arterial blood flows during activity were calculated by summing venous and saliva flows. Basal arterial flows were assumed to be the same as basal venous flows, an assumption supported by the data of Barcroft and Kato and of Tatibana. The superbasal arterial flow to the submaxillary gland during activity could now be calculated.

It was found that in 75 cases of electrical excitation of the chorda for periods of about ten minutes the ratio of superbasal blood flow to saliva flow had an average value of 12.6 with a standard deviation of the mean of ± 1.1; in 21 similar periods with pilocarpine as stimulant the value of the ratio was 4.5 ± 0.5. In figure 4 the superbasal arterial flow has been plotted on the abscissa against the saliva flow on the ordinate for experiments with electrical excitation of the chorda and with pilocarpine stimulation. On the same graph have been drawn straight lines with slopes

TABLE 1

Blood and saliva flows of submaxillary glands with chorda and pilocarpine stimulations

AUTHOR	ANIMAL	STIMULANT	RESTING		STIMULATED		
			Arterial blood flow	Venous blood flow	Arterial blood flow	Venous blood flow	Saliva flow
			ml./min.	*ml./min.*	*ml./min.*	*ml./min.*	*ml./min.*
Barcroft, 1900	Dog	Chorda			13.2	12.0	1.0
Anrep and Cannan, 1922	Dog	Chorda		1.44		3.88	0.4
Anochin, Goldberg and Samarina, 1930	Dog	Chorda		1.16		3.02	
McClanahan and Amberson, 1935	Cat	Chorda				3.82	0.16
Tatibana, 1936–38	Dog	Chorda	1.81	1.81	14.26	12.70	0.95
Present author	Cat	Chorda		0.22*		3.77‡	0.25¶
Barcroft and Kato, 1916	Dog	Pilo.	1.40	1.40	4.66	4.20	0.36
McClanahan and Amberson, 1935	Cat	Pilo.				1.43	0.27
Tatibana, 1936–38	Dog	Pilo.	1.33	1.33	4.61	3.93	0.46
Present author	Cat	Pilo.		0.22†		4.78§	1.11‖

* Average of 82 determinations, with standard deviation of the mean of ± 0.01.
† Average of 18 determinations; ± 0.01.
‡ Average of 75 determinations; ± 0.38.
§ Average of 21 determinations; ± 0.75.
¶ Average of 75 determinations; ± 0.04.
‖ Average of 21 determinations; ± 0.18.

calculated from the average values of the blood to saliva flow ratios. These lines can be seen to fit the two groups of points reasonably well, indicating in both cases a direct proportionality between extra blood flow and saliva production as found by Gesell (1919) in the case of chorda stimulation. The fact that the line for pilocarpine stimulation is higher than that for chorda excitation furnishes proof that pilocarpine stimulation of the submaxillary gland of cats produced a greater saliva flow for a given blood flow than did electrical stimulation.

From table 1 it is possible to calculate values of the ratio of mean superbasal blood flow to mean saliva flow for the dog in some cases. Tatibana's

data yield values for this ratio of 13.1 with chorda stimulation and of 7.1 with pilocarpine. The data of Anrep and Cannan yield a value of 7.1 for the blood to saliva flow ratio with chorda stimulation, while from the figures of Barcroft and Kato comes a value of 9.1 with pilocarpine. Since Anrep and Cannan say in their paper that they tried to keep blood flow low, it is possible that their data are not representative. It seems safe to conclude, therefore, that in both dog and cat pilocarpine stimulation of the submaxillary gland produced a greater secretion of saliva for a given blood flow than did chorda stimulation.

The higher saliva production with a given blood flow after pilocarpine injection may account in part for the effect of pilocarpine on the potassium content of the submaxillary gland (Wills and Fenn, 1938). It was found that as the output of potassium in the saliva increased the submaxillary

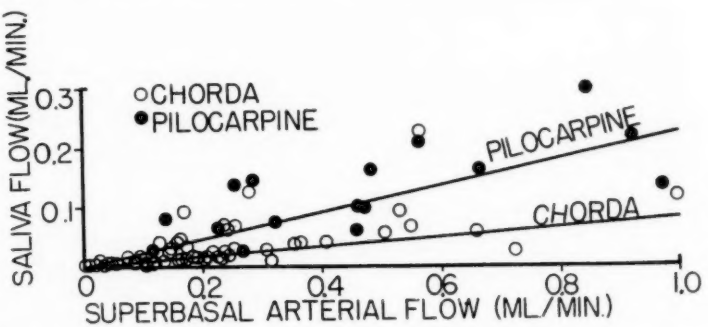

Fig. 4. Changes in the rate of saliva secretion with changes in the rate of superbasal arterial flow for submaxillary glands stimulated through the chorda or with pilocarpine.

gland was able to keep its potassium content normal if the stimulation was effected through the chorda tympani, while with pilocarpine stimulation the potassium concentration in the gland decreased rather markedly. Certainly such a difference between these two types of stimulation would be expected to show up if the potassium supply from the blood in the former case were potentially several times greater than in the latter, as now seems evident.

Langstroth, McRae and Stavraky (1938) have reported that, with electrical stimulation, at rates of saliva flow below 0.17 ml. per min. the concentration of potassium in the saliva increased with decreasing rate. At greater velocities of flow the potassium concentration was constant. A number of values for saliva potassium from the foregoing experiments are plotted against the rate of secretion in figure 5, and will be seen to conform roughly to the curve of Langstroth, McRae and Stavraky. How-

ever, it seems that the point of inflection comes at about 0.03 ml. per min. rather than at 0.17. The data of the other authors plotted on the same graph (as solid squares) seem to fit this curve almost as well as they do that in the original paper. The saliva secreted after pilocarpine stimulation apparently followed the same rule as that produced by chorda stimulation, so far as can be judged from the few data available. From this graph it can be seen also that at very low rates of secretion there was an apparently greater output of potassium by the submaxillary gland than might have been expected from the rate of secretion. This might mean that at these

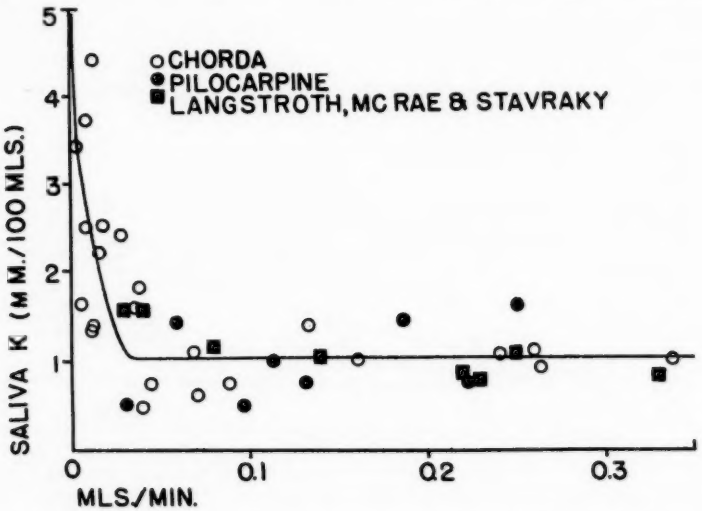

Fig. 5. Changes in the potassium concentration in saliva with changes in the rate of saliva secretion with electrical and pilocarpine stimulations of the submaxillary gland.

low rates the saliva was made up of a greater proportion of intracellular water, with a high potassium content, than at higher rates. Or it might mean that there was reabsorption of water without potassium in the ducts, as suggested by Langstroth, McRae and Stavraky (1938). It seems impossible at present to decide which of these two notions is the more correct one.

SUMMARY

The optimal frequency for electrical stimulation of the submaxillary glands of cats through the chorda tympani was about 9 per sec. Long-continued stimulation of the gland gave a series of changes in rate of secretion analogous to the changes in tension of a muscle under similar conditions.

Pilocarpine stimulation of the submaxillary gland produced a greater saliva flow for a given blood flow than did chorda stimulation. This is believed to explain in part the exhaustion of the potassium content of the gland during secretion after pilocarpine administration.

The potassium concentration of the saliva was found to increase markedly at rates of secretion below 0.03 ml. per min. This could be due either to reabsorption of water from the ducts or to a greater proportion of intracellular water in the saliva.

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THE KINETICS OF LUNG VENTILATION

An Evaluation of the Viscous and Elastic Resistance to Lung Ventilation with Particular Reference to the Effects of Turbulence and the Therapeutic Use of Helium

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The amount and nature of the work done in ventilating the lungs has not been studied extensively. In general, interest has been centered upon the elasticity of the lungs or the state of constriction of the bronchi and bronchioles. In some instances intrapleural pressures have been measured but ordinarily not simultaneously with the volume changes during respiration. Wirz (1), however, has published results of a few simultaneous recordings of pressure and volume changes and has estimated the work involved in lung ventilation in the intact animal breathing air. His attention was diverted to the study of lung elasticity and Neergaard and Wirz (2, 3) described simultaneous measurements of intrapleural pressure and respired air velocity. They attempted to estimate lung elasticity by measuring intrapleural pressures at zero velocities of air movement at the ends of inspiration and expiration respectively. This difference was found later by Paine (5) to give sometimes negative and sometimes positive values for the "elastic index" of the lungs. Although this result may be due in part to inadequacy of methods used for recording pressure and velocity simultaneously by all of the above-mentioned workers it might also be real and be due to hysteresis effects in pathological lungs. Christie and McIntosh (4) also studied intrapleural pressures and total respiratory volumes but did not estimate work. They were primarily concerned with measures of elasticity of the human lung, as was Paine (5), who compared the reliability of the methods of Neergaard and Wirz with that of Christie and McIntosh and found the latter to differentiate normal and pathological lungs more satisfactorily. Paine published records of stimultaneous intrapleural pressure and respired air velocity changes in respiration in normal and pathological subjects, to which reference will be made later.

Bayliss and Robertson (6) reattacked this general problem and developed a method for measurement of the work of artificially ventilating the lungs of experimental animals. We have employed a modification of their

method in this study which was undertaken primarily to ascertain the mechanism of action of helium mixtures in altering the work of breathing.

Barach (7) in 1934 introduced the use of a mixture of 80 per cent helium and 20 per cent oxygen in the treatment of obstructed breathing. Since then a number of papers have been published which discuss helium-oxygen therapy (8). The advantages of such mixtures in cases of tracheal or laryngeal obstruction are reported to be immediate and dramatic. Prolonged use of helium mixtures is also reported to relieve bronchial asthma, although here the action is not so rapid. It might have been supposed that the greater ease of movement of helium mixtures over nitrogen mixtures would be due to a lower viscosity. However, the viscosity of helium is actually about 10 per cent greater than that of nitrogen (see table 1). Another explanation has been given by Barach (7) who says, "since *work* is in general proportional to the density, the pressure required to move

TABLE 1

Gas constants calculated for 37° from data in the handbook of chemistry and physics

	N POISES	N RELATIVE TO AIR	d	d RELATIVE TO AIR	N/d	N/d RELATIVE TO AIR	MEAN FREE PATH
			grams/cm.^{3}				*cm.*
Air	189×10^{-6}	1.00	114×10^{-5}	1.000	1.66×10^{-1}	1.00	
N_2	187	0.99	111	0.975	1.68	1.01	8.2×10^{-6}
O_2	214	1.13	126	1.105	1.70	1.02	8.8
H_2	100	0.53	7.9	0.0693	12.65	7.61	15.4
He	208	1.10	15.7	0.138	13.24	7.97	33.1
80 He-20 O_2	210	1.11	37.8	0.332	5.56	3.35	

helium-oxygen mixtures in and out of the lung, should be decidedly less than nitrogen-oxygen mixtures." This statement is not adequate as a generalization since it ignores viscance. He also pointed out (9) that passage of air into normal lungs is practically effortless, and that substitution of helium for nitrogen in gas mixtures produced no change in intratracheal or intrapleural pressures in normal breathing. He showed, however, that when there is an obstruction in any part of the air way, increased gradients become necessary to move air past the obstruction. In 1936 and 1937 (10, 11) he demonstrated a reduction in pressure gradients under certain conditions when a helium-oxygen mixture was substituted for air.

It is possible that the rate of diffusion of CO_2, which is faster in helium-oxygen than in air, might be a contributory factor in the therapeutic value of helium, since a smaller tidal volume would be required to carry off the CO_2 if its rate of diffusion in the alveolar ducts were a limiting factor. Attention has also been drawn to the fact that diffusion of a gas is inversely

proportional to the square root of the density (7, 8). However, so far as we can ascertain, little or no attention has been paid to the conditions of gas movement in the lungs with regard to whether the movement is by Poiseuille flow, diffusion, or turbulent flow. Such information is essential before one can determine the mechanism by which helium mixtures facilitate air movement under specific circumstances.

METHODS. Dogs, ranging in weight from 12 to 16 kgm., were anesthetized with sufficient nembutal to produce medium surgical anesthesia. The animal was placed on its back, the trachea cannulated and the tracheal cannula connected to a respiration pump. Where experiments were done with a closed thorax, curare (Merck) in 0.9 per cent NaCl was injected until the animal showed no spontaneous breathing movements when the pump was stopped for 30 seconds. The chest was opened by means of a mid-line incision taking care to produce as little hemorrhage as possible. The external mammary arteries were ligated and cut, and the chest walls retracted.

The respiration pump was one which has been used in closed circuit oxygen consumption measurements on heart lung preparations. The piston has a bore of 6.35 cm., a maximum stroke of 8.56 cm. operated by a 16.80 cm. piston rod on an adjustable eccentric. The maximum stroke was 269 cm^3. The pump was driven by a $\frac{1}{4}$ H.P. electric motor through an adjustable friction wheel and reducing gears so that the duration of the stroke could be varied between 3 and 12 seconds. The pump was arranged to close an electrical circuit actuating an electromagnet causing a spot of light to be reflected onto the camera precisely at the bottom of each stroke. (Fig. 1 shows diagrammatically the connections used.) Between measurements the pump was arranged to work through suitable valves to maintain respiration.

When measurements were being made, both valves were by-passed by removing hemostats. Half a stroke later the intake tube was clamped with another hemostat close to the pump and the exhaust tube was clamped. The two last mentioned hemostats were applied as nearly synchronously as possible at the top of the stroke corresponding to maximum expiration. A T-tube between the constriction shunt and the pump led to the recording manometer. When a constriction was used, it was placed in parallel with the tubing between the manometer lead and the exhaust T-tube so that all the air moved could be shunted through the constriction by clamping the main line. All rubber and glass tubing used, except for the manometer connection and the constriction, was 1.5 cm. in diameter. All joints were sealed with glycerine.

A glass spoon manometer (12) about 3 cm. in diameter was arranged to reflect the image of straight filament onto a moving paper camera. A timer was arranged to mark tenths of a second by illuminating the camera

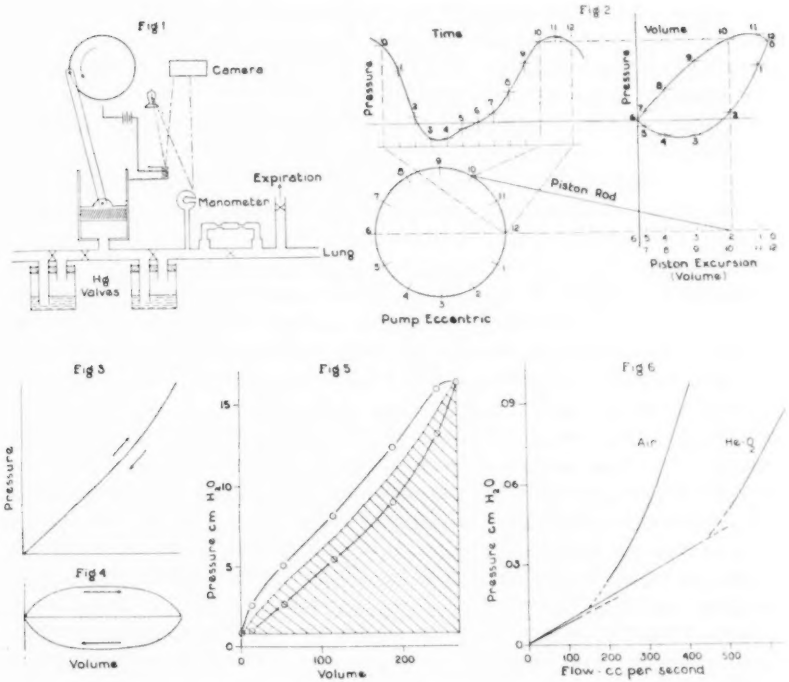


Fig. 1. Diagram of the respiration apparatus. Points where hemostats were applied are marked X.

Fig. 2. Diagram of the method used to construct pressure volume curves from pressure time records and the geometrical constants of the pump.

Fig. 3. Pressure volume diagram for a theoretical elastic system without viscance.

Fig. 4. Pressure volume diagram for a theoretical viscous system without elastance.

Fig. 5. Pressure volume diagram for air in a dog lung. The area of the closed loop represents viscous work and the shaded area represents the elastic work of inspiration.

Fig. 6. Viscous and turbulent flow. These curves were calculated from the equations:

$$P_s = \frac{128\ \eta\ L\ Q}{\Pi\ D^4} \qquad P_t = \frac{64\ k\ d\ Q^2\ L}{\Pi^2\ D^2}$$

P_s is the pressure in streamline flow, P_t is the pressure in turbulent flow, n is the viscosity, d the density, D the diameter of the tube, L its length, and Q the rate of flow of gas in centimeters^3 per sec. D was taken as 1 cm. and L as 10 cm. k was given the mean value 0.008. P is given in dynes and has been converted to centimeters of water by the factor 0.00102.

through a slotted disk, rotated by a synchronous motor. The manometers were calibrated by means of a water-filled U-tube and were linear with an error of less than 5 per cent over the useful range. A typical manometer had a natural frequency of 68 cycles per second. Manometers of this type are superior to rubber tambours since they have a linear response to pressure and can be made with an extremely small inertia.

Figure 2 shows diagrammatically the method of analyzing a record. The space between two marks, indicating full inspiration, was divided into 12 (or 24) intervals of equal duration corresponding to 30° (or 15°) rotation of the pump. The pressures corresponding to each of these times were measured making use of a graph of pressure against deflection for conversion. The volume displaced by the pump at each time interval was determined graphically as shown in figure 2 and was subsequently checked by trigonometric calculation. The relation of volume to time was not sinusoidal because of the construction of the pump. It deviated 7 per cent from a true sine wave at mid-stroke for the largest volume. The pressure was then plotted against volume to produce a loop which was closed if the pressures at two consecutive maximal inspirations were identical. Areas were obtained from the records by means of a planimeter. In case the loop was not closed, the area was corrected by the area of a triangle having as its base the difference of the final pressures, and as its altitude the total volume of the pump. This is practically equivalent to adding a linearly changing value to all points on the curve so that the ends coincide. In no case did we plot a curve which failed to close by more than 0.5 cm. H_2O and the correction never exceeded 5 per cent of the total area. Figure 8 is a photograph of four parts of a record which have been plotted as pressure volume curves in figure 7.

Results and discussion. a. *Theoretical considerations.* Bayliss and and Robertson (6) introduced the terms viscance and elastance. These quantities differ from viscosity and elasticity in that they are properties of a system and not properties of a substance. For example, the viscosity of water at a given temperature is a constant but the viscous resistance to a unit velocity of flow of water, or the viscance, depends on the dimensions of the tube through which the water is flowing. Similarly the elasticity of a gas obeys Boyle's law but the pressure produced in a gas for unit change of volume, or the elastance, is a function of the volume of the gas.

The work of ventilating the lungs is eventually all dissipated as heat produced by the viscance of the air and the tissues of the body. However, during inspiration some of the work is done against elastic forces. The potential energy stored by these elastic forces, including work done in lifting parts of the body against gravity, is partly used during expiration to move the air and tissues against their viscous resistance. With active expiration, additional work is supplied by the respiratory muscles, whereas,

if expiration is restrained, some of the energy stored will be returned to the respiratory muscles. Energy returned to a muscle is converted into heat and wasted and in addition the muscle must do extra work (13). Work can also be stored during the early phases of inspiration and expiration as kinetic energy of motion of the air and tissues; this energy is liberated during the later stages and has the effect of reducing the maximal elastic pressure in the lung. The magnitude of this inertia effect in the air is, however, very small because of the small masses involved and amounts to a maximum pressure of less than 0.5 mm. of water at the maximum frequencies and pump volumes used in this work. In any case, since the energy used to impart momentum to the gas and lung tissues at the beginning of the stroke is all regained at the end of the stroke, it is not wasted and requires no net energy expenditure by the driving mechanism.

The pleural cavity, when the chest is relaxed, is normally at a negative pressure (about 5 cm. H_2O below atmospheric). During inspiration this negative pressure increases, and at its peak may be increased by 5 to 25 cm. H_2O (14). We have assumed that it will make no appreciable difference whether inflation is produced by lowering the pleural pressure or raising the tracheal pressure; if the differences in pressure are the same, the effects would be the same so long as we can consider the air to be incompressible. Since 25 cm. of water is about 2.5 per cent of an atmosphere, the compression or expansion will be only 2.5 per cent and can probably be neglected for this work. We recognize that time is involved in movement of gas during pressure equalization and that for this reason a slight difference between positive tracheal pressure and negative intrapleural pressure inspiration is to be expected.[1]

If the trachea is connected to a simple valveless pump and pressure in the trachea is recorded, it is possible to evaluate the viscous work of ventilation as well as the work stored in elastic tissues during inspiration. To do this the pressure is plotted on a vertical axis and the volume of air displaced by the pump on a horizontal axis for one complete respiratory cycle. To understand the meaning of curves of this sort, it is useful to consider some elementary models.

If the lung be replaced by a perfectly elastic container, the pressure will be determined only by the volume of the pump and the inspiratory and expiratory curves will be identical as in figure 3. In the ideal case where Hooke's law is obeyed this curve will be a straight line. A practical model of this case is a large rigid bottle where the elasticity is furnished by the gas itself.

[1] The differences between positive and negative lung pressures become extremely significant, of course, when their effects on the blood supply of the entire thorax are considered (15).

If the lung is replaced by a non-elastic accordion or bellows connected to the pump through a constricted tube, the pressures will depend on the velocity of air in the system. This is zero at the minimum volume, increases to a maximum during inspiration, and returns to zero again at maximum volume. During expiration the velocity follows a similar course returning to zero at the end of the stroke. The pressure will vary as shown in figure 4. The area of the loop measures the integral of PdV, which is work, and is expressed in gram centimeters if the pressures are measured in grams per square centimeter and the volume in cubic centimeters (1 gram centimeter = 0.0000234 gram calories).

The lung can be compared to an elastic accordion containing some viscous resistance. Figure 5 shows a curve obtained from a normal dog lung. It can, as a first approximation, be considered as the sum of the curves 3 and 4. The area of the loop again represents the work done against viscous resistance in the lung. In order to determine how much work has been stored elastically during inspiration, we may assume that the viscance at any volume depends only on the velocity of flow and not on its direction. Then the theoretical curve of elasticity will be the median line of the loop and is shown dotted in figure 5. The work stored elastically during inspiration is then the area between this curve and the axis of zero pressure which is indicated by shading. It is also equal to the entire area above the axis in figure 5 minus half the area of the loop. Zero pressure must be taken as the pressure of minimum volume of the lung for the purposes of these calculations. Although the assumption of symmetrical distribution of viscous pressures does not hold in all cases, as will be shown, the errors are probably not very great when the work of the whole cycle is considered.

The viscous work of ventilating the lungs, which may be defined as the dissipative work of ventilation, can be considered to consist of two parts. One part is contributed by the air and represents the air viscance in the airways of the lung. The other part is contributed by the tissues and contains the viscous resistance to movement of the tissues as well as any components possibly introduced by muscular action within the lung.

b. *Modes of gas movement.* Gaseous movement can occur in three ways, by diffusion, by streamline or Poiseuille flow, and by turbulent flow (16, 17). When the orifice through which gases are flowing has a diameter comparable to or smaller than the mean free path of the gas, diffusion will be the dominant form of gas movement. The rate of diffusion of a gas is proportional to the pressure and inversely to the square root of the molecular weight. In air or nitrogen the mean free path of the molecules is of the order of 0.0001 mm., while helium at atmospheric pressure has a mean free path of 0.003 mm. (table 1). The diameter of the smallest tubes occurring in the lung is 0.1 mm. (19) so that it seems unlikely that diffusion

is directly involved in actual mass movement of air in the lung, as suggested by Barach (7). To test this hypothesis further we compared the rate of escape of a fixed volume of air and helium at the same pressures through various resistances. The gas was contained in a gas pipette over saturated NaCl. The resistance was attached to the top of the bulb and flow was produced by raising the leveling bulb producing a maximum pressure of 30 cm. of water. When a glass capillary about 0.1 mm. bore was used, the time of escape of air was about 10 per cent less than the time for helium. This is what would be expected, since air has a slightly lower stream-line viscosity than helium (see table 1). Since a straight capillary is not a very satisfactory model for the lung, we also tried a long glass tube, 5 mm. in inside diameter, packed with NaCl crystals which varied from 0.10 to 0.15 mm. on a side. This provided a number of contorted passages less than 0.1 mm. in diameter which is, according to Miller (19), smaller than the smallest bronchioles in the lung. This model showed indistinguishable rates of escape of air and helium. It is possible that there was some diffusion in this model, but since the rate of diffusion of helium is 2.7 times the mean rate of diffusion of air, there can not have been more than 5 per cent of the movement by diffusion. It can be concluded that diffusion does not contribute materially to the mass movements of air in such systems. As a further check a sintered glass filter having pores of the order of 0.03 mm. was used. In this case helium escaped 1.43 times as fast as air. There was still some indication of Poiseuille flow since this ratio is smaller than would be expected if diffusion were the only form of gas movement.

Streamline flow of gas obeys Poiseuille's law. The pressure is proportional to the rate of flow and the viscosity of the gas. The only gases with viscosities sufficiently different from air to be useful in studying streamline flow are the inflammable gases, hydrogen, methane, and ethylene which have lower viscosities than air, or the very rare gases, neon and krypton, with higher viscosities. Hydrogen is probably the best choice and was used by Bayliss and Robertson (6) in a mixture with 20 per cent oxygen. This mixture is highly explosive and we have employed it in only a few experiments discussed later.

Turbulent flow of fluids obeys a different law from either diffusion or streamline flow. The pressure necessary to produce turbulent flow varies approximately as the square of the velocity of fluid moved, and as the density of the fluid (16). There is a certain critical velocity of flow below which the flow is streamline and above which streamline flow is unstable and will become turbulent if disturbed. This critical velocity depends upon the shape of the conducting system. For a given geometrical system, however, the critical velocity of flow is proportional to the viscosity divided by the density of the fluid. Although these laws have been developed for

incompressible fluids, they hold to a high degree of accuracy for gases as well. Helium with its slightly higher viscosity and much lower density will flow faster than air before its flow becomes turbulent. At velocities of flow where air is turbulent the pressure, necessary to maintain the flow of helium-oxygen mixture, will be less than the pressure to maintain the same flow of air. Figure 6 shows the calculated pressures for 80 per cent He, 20 per cent O_2, and for air in the same system. The viscosity of the helium-oxygen mixture is taken as 1.11 and the density 0.33, both relative to air.

It will be seen that below the critical velocity and for a small distance above it, the pressure on the air is 10 per cent less than on the helium-oxygen mixture. But above this point the air requires more pressure than the helium-oxygen mixture. The ratio of pressures necessary for equal flow increases until the helium becomes turbulent. Above this velocity helium-oxygen mixtures require 0.33 of the pressure for an equal flow of air.[2]

Breath sounds which are caused by air in vibration indicate that the flow of air is turbulent, at least at the point of origin of the sounds. Therefore it is to be expected that noisy labored breathing would be relieved by the use of helium-oxygen mixtures, provided that a large part of the effort of breathing is being expended against gas movement. The critical velocity of flow depends inversely on the diameter of the conducting tubes, other things being equal. Now the bronchioles of the lungs become progressively smaller with each division, yet their total cross sectional area increases (20). Gas flow must therefore be slower in the distal bronchioles. Both reduced velocity and smaller diameter of the conducting tubes will tend to eliminate turbulence in the distal tubules so long as the latter are not partially occluded, as by mucus. Sharp flexures in the conducting tubes will, of course, produce turbulence at lower velocities, but the air ways of the lungs show very smooth junctures of a type which should discourage turbulence. Turbulence is therefore probably confined to the air passages in the head, the larynx, the trachea, and the larger bronchi. Roher (17) comes to the same conclusions from measurements on models. Figure 6 shows that the critical velocity of air flow in a 1 cm. tube is 130 cc. per second, which is similar to the conditions existing in the secondary bronchi of a dog, or the tertiary bronchi of man. The pressure drop necessary to maintain this flow in a tube 10 cm. long is 0.1 mm. of water. It would seem unlikely, therefore, that turbulence could contribute very much resistance to quiet breathing in normal lungs. Also, these considera-

[2] The above simplified discussion of turbulence is derived from Franklin and Grantham (16). Actual critical velocities depend to some extent on the smoothness of the conducting tubes, and the value of k used in figure 6 is not a true constant. The qualitative conclusions, however, are unaltered by a more exact treatment.

tions show that an obstruction in the proximal bronchi or the trachea is much more likely to produce resistance by turbulent flow alone, and, in fact, cases of just this sort are said to receive the most immediate benefit from the use of helium-oxygen mixtures. Schultz and Jordan (21) showed that, in the guinea pig, anaphylactic shock caused constriction in the secondary bronchi. Contractions which occur at this level would be much more likely to cause turbulence and abnormal breath sounds than contractions of the terminal bronchioles.

c. *Turbulence and the effects of helium.* It is possible to determine whether turbulence plays any significant rôle in the work of ventilating the lung by substituting a mixture of 80 per cent helium—20 per cent oxygen for air. We have done this experiment 21 times on 4 dogs with normal airways and have never found any significant difference of viscous work between the two mixtures. It is usually impossible to detect any difference whatever in the curves, provided that the filling of the pump with air and with helium is done at the same pressures.

However, when a constricted tube, designed to produce turbulence as shown in figure 1, is placed in series with the trachea, the pressures accompanying ventilation of the lung are altered and the viscous work is increased. When a helium-mixture is substituted for air this work is markedly decreased. This fact has been verified in numerous trials. Figure 7 shows the results of a typical experiment upon the effects of air and helium through the same constriction, as well as a record without the constriction which shows identical work for air and helium. The original pressure tracings from which these curves were drawn are shown in figure 8. The effect of substituting helium for nitrogen is apparent in the pressure tracings as well as in the pressure volume diagrams. However, the area of the loop of the pressure-volume diagram is a direct measure of the work done during the cycle and is therefore directly correlated with the effort of breathing. It is possible to have large changes in the area of the pressure-volume diagram with only negligible changes in the maximal and minimal pressures.

These observations prove that air viscance in obstructed larger airways is diminished by substituting helium for nitrogen. Since helium has a higher streamline resistance but a lower turbulent resistance than nitrogen it is obvious that the lower work in moving the helium mixture is due to its higher critical velocity and lower turbulent viscosity.

This effect is very large when constrictions are placed in the upper airways. In order to test the effect of airway constriction at other points upon turbulence, pilocarpine, 1 mgm. per cent in physiological saline, was administered intravenously to a dog after control studies of ventilation work had been made under standard conditions. An initial dose of 10 mgm. caused profound cardiac slowing and injection was continued with a continuous injector at the rate of 0.5 mgm. per minute. Pressure-

volume curves taken before and after injection showed a 60 per cent increase in elastic work. Substitution of helium-oxygen for air after pilo-

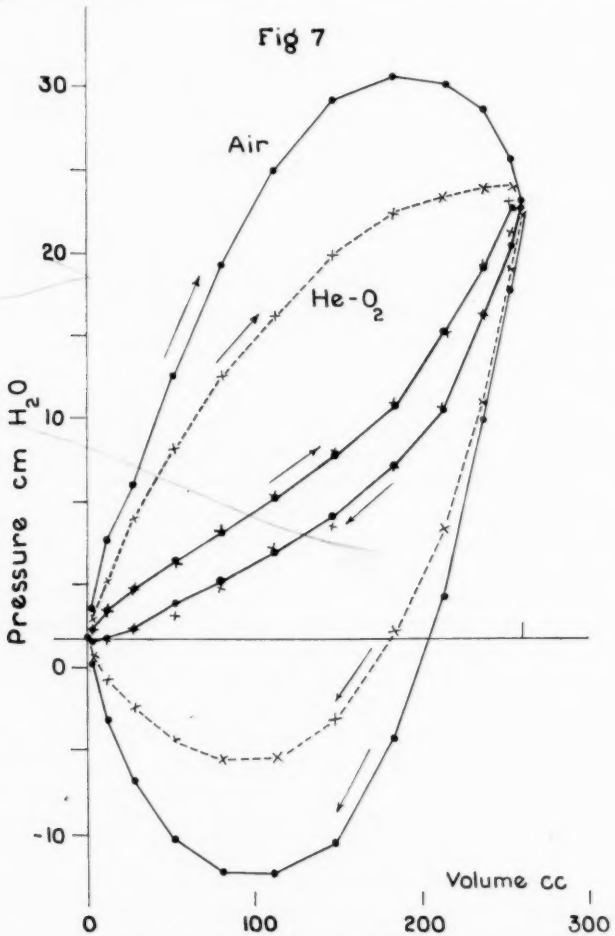
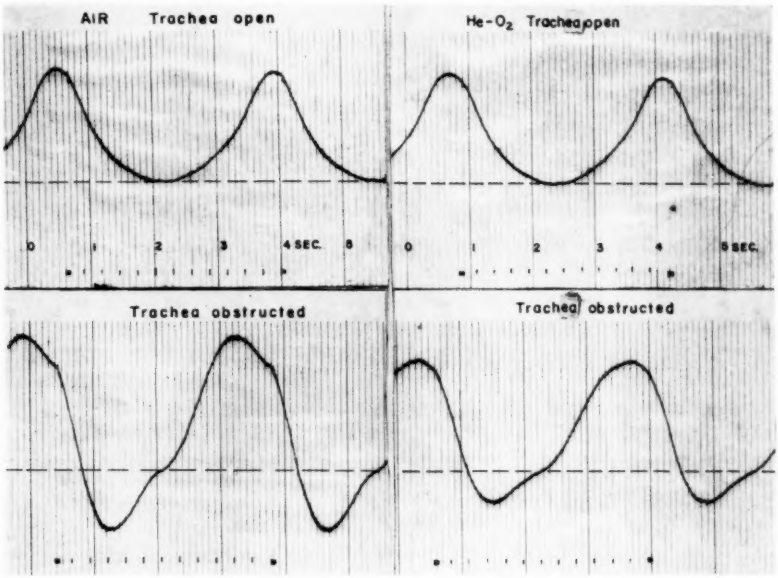


Fig. 7. Pressure volume diagram with and without tracheal obstruction. The central loop marked with • and + represents air and helium oxygen with no obstruction. The outer loop marked with • represents air with a constriction as shown in figure 1. The middle loop marked with + represents helium-oxygen through the same constriction.

carpine left the elastic work unchanged but decreased the viscous work 10 per cent (table 2). These results confirm the observations of Bayliss and Robertson (6), who found small alteration in gas viscance by sub-

stituting hydrogen for nitrogen after pilocarpine administration. Both sets of observations indicate that increase in gas viscance is a small factor



(*a*) (*b*)

Fig. 8. Time pressure records from which figure 7 was determined. The left margin of the solid black marks on the lower part of each record represents the instant the pump reached the bottom of its stroke corresponding to maximum inspiration.

TABLE 2

Work of ventilation before and after pilocarpine – Tidal volume 261 cc., respiration rate 20 per min.

	VISCOUS WORK V	TOTAL WORK T	ELASTIC WORK $E = T - V/2$
	gm. cm.	*gm. cm.*	*gm. cm.*
Before drug:			
Air or $He-O_2$	1980	3152	2162
After drug:			
Air	1704	3980	3028
$He-O_2$	1540	3820	3050

in the increased resistance to breathing following pilocarpine, and that tissue elastance and viscance are more important factors.

Since without obstruction to airways we have found turbulent gas vis-

cance to be below the limit of detection we can conclude that gas viscance in normal lungs follows Poiseuille's law and should vary linearly with velocity. Tissue viscance is a more complex function, but it is in part composed of simple fluid viscance. Other factors undoubtedly enter in and it seemed to us to be possible that the importance of those other factors which are connected with structural plasticity might be evaluated by measuring the work of ventilating the lungs at various speeds.

A number of experiments were therefore done at slow and fast pump speeds. Three of these which seem to be typical and which were uncomplicated by breathing movements were measured and the results are listed in table 3. While the results show rather wide variations in different animals and under various conditions, it is evident that the reduction in

TABLE 3

Work of ventilation in relation to velocity—Tidal volume 269 cc.

EXPERIMENT	VISCOUS WORK, V	TOTAL WORK, T	ELASTIC WORK, $T - V/2$	DURATION OF STROKE, S	FREQUENCY, $f = 1/S$	$V_1 - V_0 = f_1 \frac{V_1 - V_2}{f_1 - f_2}$	V_0	$\frac{V_0}{V_1} \times 100$
	gm. cm.	*gm. cm.*	*gm. cm.*	*seconds*		*gm. cm.*	*gm. cm.*	*per cent*
A_1	918	1920	1461	2.87	0.348			
A_2	608	1840	1536	9.9	0.101			
$A_1 - A_2$	310				0.247	437	481	52
B_1	600	2155	1855	2.85	0.351			
B_2	534	2110	1843	10.75	0.093			
$B_1 - B_2$	66				0.258	90	510	85
C_1	790	1855	1490	2.90	0.345			
C_2	560	1684	1424	4.80	0.208			
$C_1 - C_2$	230				0.137	575	215	28

total viscous work is not proportional to the frequency of the pump. There must be some fraction of the viscous work of ventilation which is relatively independent of velocity. This work can not be in the gas nor in the viscance of ideal fluid in the lung tissue for the reasons outlined above. It may be deduced that tissue resistance related to structural plasticity accounts for a large fraction of the viscous work. We have calculated the magnitude of this component of tissue resistance on the assumption that the Poiseuille viscous resistance of gases and liquids in the lung is linearly proportional to frequency and is added to the plastic resistance. The results as shown in columns 8 and 9 of table 3 indicate clearly that a considerable fraction of the viscance does not vary linearly with velocity. There is, of course, no *a priori* reason to suppose that the plastic resistance will be a constant quantity or a constant fraction of the

total viscous resistance. Bayliss and Robertson (6) found essentially the same thing; a component of viscance which was largely independent of frequency. They also report that the total tissue viscance at 18 cycles per minute accounted for about two-thirds of the total viscance. Our results confirm theirs to within the accuracy of the determinations.

d. *Hysteresis effects and elasticity.* We investigated the effect on the pressure-volume diagram of altering the residual volume of the lung by connecting a 100 cc. syringe to the line so that air or gas mixtures could be added or removed. Figure 9 shows the effect on the diagram when two successive additions of 100 cc. were made. The diagrams shown here were taken 3 to 6 seconds after injecting the air into the system, and each cycle required 2.8 seconds. The abscissal values for the second and third loops were taken as the respective pump volumes plus the amount injected. If the lungs behaved as a simple elastic system, the median lines of the three loops would all lie on the same line. Actually, the pressure produced by adding 100 cc. 6 seconds earlier is not as great as the average pressure when the pump has put 100 cc. into the lungs which required only 0.65 second at the speed employed. The following experiment was performed to elucidate the phenomenon further. The pump was stopped and 100 cc. of air was injected every 5 seconds, the injection taking 0.6 second, until 500 cc. had been injected, then 100 cc. was removed every 5 seconds. Pressure was continuously recorded, and it was found that there was a rise of pressure during the injection, followed by a gradual fall, until the next injection was made. Figure 10 shows the result of one of these experiments, done on a living dog with the chest open. Practically all the drop in pressure at constant volume took place in one second. It will be seen that there is a hysteresis effect in that the pressure on deflation is less than the corresponding pressure on inflation. This hysteresis effect has the same influence on the pressure-volume diagram as does viscance when the pump is moving continuously. However, in this experiment there was no gas movement during the equilibration process. A number of step inflation experiments were done on the lung of a dog *in situ* soon after death. No precautions were taken to keep the temperature above that of the room. The dying lung shows a much greater hysteresis effect than the living lung (fig. 11), and part of the decay of pressure continues after the first second.

This effect could possibly be qualitatively explained by assuming that the elasticity of the lungs is in two sets of chambers, separated by a high viscance. Then if air is rapidly introduced into the first set of chambers, this would cause a high pressure which would fall as the air flows into the second set. However, when we consider the size of the available chambers and tubes, the time required to reach equilibrium seems much too long. It is possible to rule out this hypothesis by substituting pure hydrogen for the air in the lung. Hydrogen with its lower viscosity and

much higher critical velocity should decrease the time necessary for equilibrium. Figure 12 shows the effect of step inflation of the same lung with hydrogen only 400 cc. was injected, and the resting volume must have been somewhat larger since the curve does not exactly coincide with figure 11; but the difference is not significant. The fall in pressure immediately following the injection of hydrogen is in fact somewhat larger than for air.

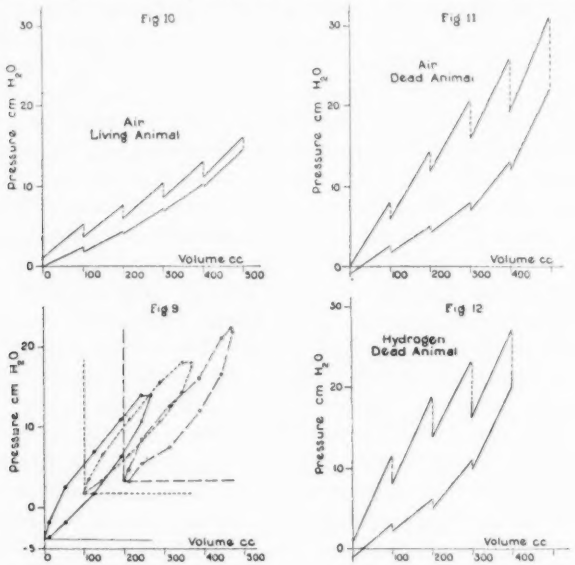


Fig. 9. Pressure volume diagram before and following two separate additions of 100 cc. of air.

Fig. 10. Pressure volume diagram for air in a live lung with the chest open. The change in pressure at constant volume occurred during about 1 second after each change in volume.

Fig. 11. Pressure volume diagram for air in a dead lung. The change in pressure during the first second is shown as a dotted line, during the remaining 3.5 seconds as a solid line. Changing the volume took 0.5 second and was done every 5 seconds.

Fig. 12. Same as 11 using hydrogen.

The effect is not due to asphyxia of the lung by hydrogen since pure nitrogen was practically indistinguishable from air in its effect. We suggest that the higher initial reaction of the lung to hydrogen is a result of the fact that the hydrogen could be injected more quickly than air because of its lower viscous and especially turbulent resistance.

The response of the lungs to a change in pressure of the type described above is a hysteresis effect. The hysteresis or accommodation effect manifests itself as a gradual change in residual air volume following a

maximal inspiration. Christie and McIntosh (4) use such a change as an indication of "set" or plasticity which they claim occurs only in pathological and not in normal lungs, although it is a fairly common observation following exercise on a metabolism apparatus. In any case their methods would not detect small hysteresis effects such as we have measured. The much larger hysteresis effects which we found in the lungs deprived of circulation might be considered as a form of pathological response. Hysteresis has been noted in the dead lung by Hirakawa (24).

As would be expected, opening the chest reduces the elastic work of respiration. Table 4 and figure 13 show the changes in the several work fractions produced by opening the chest. The change in viscous work is variable but there is a consistent reduction in elastic work. The variation

TABLE 4

Work of ventilation with closed and open chest—Tidal volume 269 cc.

	CLOSED CHEST			OPEN CHEST			$\frac{V_1}{V_2}$	$\frac{E^1}{E_2}$
	Viscous work, V_1	Total work, T_1	Elastic work, $T_1 - V_1/2$	Viscous work, V_2	Total work, T_2	Elastic work, $T_2 - V_2/2$		
	gm. cm.	*gm. cm.*	*gm. cm.*	*gm. cm.*	*gm. cm.*	*gm. cm.*		
A	600	2155	1855	760	1885	1505	0.76	1.23
B	1000	2860	2360	520	1780	1520	1.92	1.60
C	780	2850	2460	700	2270	1920	1.11	1.28
D	620	1940	1630	720	1560	1200	0.86	1.36
E	1172	2980	2394	568	1316	1032	2.06	2.32
Mean							1.34	1.56

in work of ventilation is due mainly to the use of constant ventilation volume with dogs of differing size.

f. *Elasticity and normal breathing.* Pressure volume curves for normal breathing in the human may be calculated from the data of Neergaard and Wirz (2, 3) and Paine (5). To do this the curve of air velocity is integrated by adding the ordinates at equal time intervals to obtain relative volumes. Paine gives absolute volume changes so that the relative volumes can be calibrated. We have integrated all the curves in the papers referred to above and reproduce three of them here, figures 14, 15, 16. In this case intrapleural pressures are plotted in such a way that the curves have the opposite inclination from our other curves. They would have the same inclination if we had plotted the difference between oral pressure and pleural pressure as a positive value.

Figure 14 from Neergaard and Wirz is the only one of these curves which is similar in shape to our curves with pump respiration. Even so the

viscous work is a large fraction of the total work. Unfortunately there are no data in the original paper which will permit the calculation of either pressure or volume in absolute units. All of Paine's diagrams are more

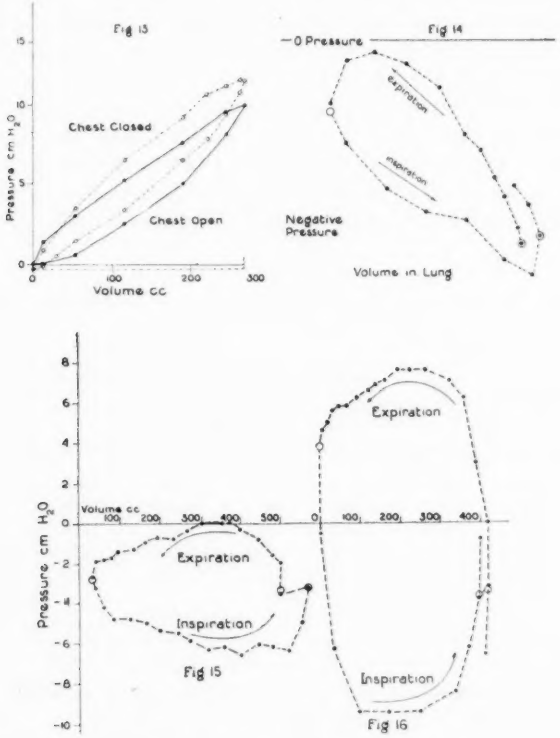


Fig. 13. Pressure volume diagrams with the chest closed and open.

Fig. 14. Pressure volume diagram obtained from the curve of Neergaard and Wirz (3) p. 61. Volume in the lung increases in arbitrary units to the right. Open circles indicate points at which the air velocity was zero.

Fig. 15. Pressure volume diagram for a patient with normal lungs obtained from the data of Paine (5), figure 4. Open circles indicate points at which the air velocity was zero.

Fig. 16. Pressure volume diagram for a patient with pulmonary emphysema. Obtained from the data of Paine (5), figure 8. Open circles indicate the points at which air velocity was zero.

nearly rectangular and the elastic component is difficult or impossible to measure with accuracy. His patients all seem to exert an approximately constant intrapleural pressure throughout inspiration and expiration. It

follows that the air velocity falls progressively as the elastic pressure in the lung approaches the intrapleural pressure since it is this difference in pressure that produces air flow. Figure 16 is a record of a patient with extensive emphysema and shows the high positive pressures necessary to produce expiration. The points are spaced at equal intervals of time and show the prolonged expiration time. Although in this case the apparent elasticity is high it is anomalous in that it indicates an intrapleural pressure greater than atmospheric at the beginning of inspiration when the air is moving into the lungs. It is most probable that the emphysematous lung has a very large plastic viscance and little if any elasticity so that the patient must use special effort to produce expiration and can not rely on the elasticity of his lungs. If this is true then helium-oxygen mixtures could produce no reduction in the work of moving equal volumes of gas in emphysema. The larger mean free path of the molecules in helium mixtures might still produce favorable effects.

CONCLUSIONS

1. The time relations of pressure and volume changes produced during the ventilation of dog lungs by a valveless pump have been measured.

2. Diagrams of these pressures against the volume displaced by the pump permit the separate evaluation of viscous and elastic resistance to ventilation.

3. The viscous resistance of the normal lung is not changed when a helium-oxygen mixture is substituted for air.

4. The viscous resistance produced by an obstruction which produces turbulence is reduced by the substitution of helium-oxygen for air.

5. It is concluded that the mode of action of helium in reducing the work of ventilation is by a reduction of turbulence in the gas flow.

6. The lung shows adaptation to changes in volume which accounts for a large part of the viscous pressure of ventilation.

7. Pressure volume diagrams calculated from data in the literature are shown for human subjects breathing naturally.

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THE INFLUENCE OF GLYCINE ON MUSCULAR STRENGTH

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Down the centuries one mark of man's prowess has been great muscular strength, and any means of increasing it has been as much sought for as the philosopher's stone. One of the most recent expedients is the addition of glycine to the diet (Chaikelis, 1941). If this does increase muscle strength, the increase would appear to be implicit in the reported rôle of gelatin, which is one-quarter glycine, in increasing work capacity on the bicycle ergometer (Ray et al., 1939). Possibly glycine, whether ingested as such or as a constituent amino acid in gelatin, has a creatinogenic action. If so, its reputed action in exercise might find an explanation, as well as its effect on muscular dystrophies (Boothby, 1934).

Most recent studies of the addition of gelatin to the diet have failed to reveal any fatigue-allaying action or any effects on work performance (Hellebrandt et al., 1940; Robinson and Harmon, 1941; Karpovich and Pestrecov, 1941; and unpublished data from this laboratory). However, the positive effects of glycine feeding on muscle strength reported by Chaikelis are so clear-cut that we decided to repeat one phase of his study.

PROCEDURE. Eight men on our laboratory staff squeezed a hand dynamometer as a test of strength of grip morning and evening for a period of eleven weeks. After the first week, when all were on their usual diet, six subjects began receiving six grams of glycine per day while the other two received placebos. No subject knew what he was receiving with the exception of S. M. H. During the tenth and eleventh weeks, one of the latter two (R. C. D.) was placed on 12 grams of glycine daily. At the beginning of the sixth week, glycine was withdrawn from two subjects who were then placed on placebos for the remainder of the experiment. Each week a 24-hour urine was collected by the subjects. The urines were analyzed for creatine, creatinine and nitrogen by methods previously described (Dill and Horvath, 1941).

RESULTS. The results of the strength measurements are the weekly means expressed as per cent of the control week. The data for the separate hands are shown for each subject (fig. 1).

Control subject R. C. D. had increased in strength measurement 8 to 10 per cent over the initial value by the ninth week. The ingestion of 12

grams of glycine daily during the following two weeks did not augment his performance. Control subject C. A. K. attained his highest level of

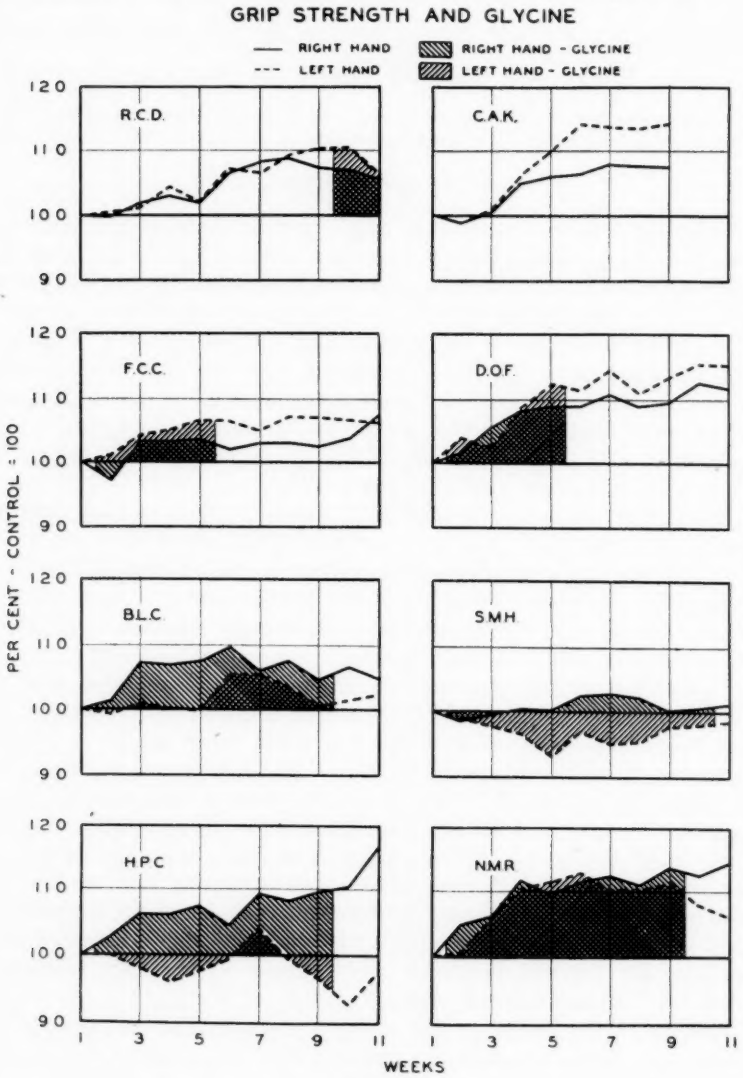

Fig. 1

improvement in six weeks and maintained it thereafter. This was roughly 8 per cent for the right hand and 14 per cent for the left.

F. C. C. and D. O. F., who received glycine for four weeks, did not improve more than control subjects. After being placed on placebos when they were withdrawn, D. O. F. exhibited during the six weeks following an actual increase, while F. C. C. remained at about the same level.

The other experimental subjects did not show greater increases in grip strength than control subjects. B. L. C.'s maximum gains were 10 per cent for the right and 6 per cent for the left hand. The grip strength of S. M. H. increased to a maximum of only 3 per cent with the right hand and showed a decrease for the left; it never reached the control level throughout the glycine period. H. P. C. had a greater right hand grip,

TABLE 1

The mean 24-hour excretion of creatine and creatinine in grams in the urine of control subjects and subjects ingesting 6 grams of glycine per day

	WEEKS									
	1	2	3	4	5	6	7	8	9	10
	Control	Placebos								
Control subjects (2)										
Creatine	0.13	0.03	0.05	0.14	0.25	0.26	0.41	0.12	0.06	—
Creatinine	2.03	1.97	1.99	2.12	2.09	1.96	2.19	2.01	1.98	—
	Control	Glycine				Placebos				After
Experimental subjects (2)										
Creatine	0.21	0.06	0.12	0.41	0.41	0.22	0.23	0.37	0.24	0.19
Creatinine	1.92	2.19	2.21	1.70	2.09	1.84	1.95	2.01	2.44	2.10
	Control	Glycine								After
Experimental subjects (4)										
Creatine	0.08	0.06	0.03	0.16	0.08	0.22	0.14	0.19	0.12	0.15
Creatinine	1.73	1.85	1.83	1.74	1.88	1.81	1.88	1.85	2.03	1.86

but a variable and usually sub-control left. He showed a spurt the second week after glycine was withdrawn. N. M. R. showed an improvement of roughly 11 and 14 per cent for separate hands.

Glycine does not increase muscle strength nor modify the course of the training curve. The changes in performance can be ascribed to training and to increased skill in using the instrument. No subject reached the mean order of strength increment recorded by Chaikelis (22–23 per cent), even though our experiment had a training factor which strengthened the grip. Chaikelis reports no intermediate tests between the initial and final ones, and records no activity which would have tended to train his subjects for this task. Ray's statement that the work performance of his subjects

declined after being deprived of an adjuvant containing glycine was not confirmed. There was no drop in the performance of the present subjects during periods of 2 to 6 weeks following cessation of glycine.

The urinary excretion of creatine and creatinine in one period of 24 hours was determined once each week, as shown in table 1. Creatinine excretion varied from week to week with possibly a slight upward trend. This was not in agreement with Chaikelis' results, where creatinine concentrations decreased roughly 30 per cent. Chaikelis' results on creatinine were obtained on urines drawn some time within six hours of a bout of exercise; the volumes were not measured. Such values indicate concentrations, not amounts. Our results, on the other hand, are in line with our previous finding that gelatin does not alter the creatinine excretion. The variation from day to day in a single subject was generally greater than the mean differences observed. In agreement with previous evidence (Dill and Horvath, 1941; Horvath and Corwin, 1941), creatine was excreted. The alterations in its excretion during the period of the study cannot be ascribed to glycine *per se*, since they were equally great when glycine was not being administered. There was a slight increase in nitrogen excretion during the period of glycine ingestion.

SUMMARY

Eight subjects were tested for strength of grip twice daily for eleven weeks. Four subjects received six grams of glycine daily for eight weeks. Two received it for four weeks and then placebos. Two others served as controls for nine weeks. One of these was then given twelve grams of glycine daily for two weeks.

The improvement in grip strength in subjects receiving glycine was no greater than that of those who were given placebos. Neither creatinine nor creatine excretion showed changes attributable to the ingestion of glycine.

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ANALYSIS OF THE INITIATION OF FIBRILLATION BY ELECTROGRAPHIC STUDIES[1]

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It has been pointed out by Wiggers (6) that ventricular fibrillation, evoked by application of a strong brief shock during the vulnerable period of late systole or of early diastole, starts with a series of 3 to 6 undulatory contractions which have many earmarks of premature beats. They are attended, in standard electrocardiogram leads, by large bizarre complexes which recur at progressively decreasing intervals and change their form in successive beats. Such complexes apparently contain a clue as to the mechanisms responsible for the induction of fibrillation. Unfortunately, however, they represent the resultant of so many ultimate electrical potentials that their further study has not seemed promising.

In this investigation, an intimate study of electrograms recorded from various small spots on the ventricular surface was primarily undertaken with the hope of elucidating *a*, the mechanisms by which strong, brief and *localized* shocks can cause a *general* disorganization of ventricular excitation necessary for the fibrillating state, and *b*, the reasons why this is only brought about, in normal hearts, by stimuli which fall during the vulnerable phase. It was soon found that this also required a restudy of single responses of the ventricles by stimuli applied at various moments of systole and diastole.

PROCEDURES. Dogs from 8 to 15 kilos in weight and of both sexes were used as experimental animals. Anesthesia was induced by intravenous administration of sodium barbital, 200 mgm. per kilo, usually preceded by a subcutaneous injection of 1 or 2 cc. of 2 per cent morphine sulfate solution. Mild artificial respiration was instituted and the chest opened by a midline incision through the sternum. The heart was suspended in a pericardial cradle and kept moist with drip Ringer's fluid. Experiments were usually completed within four hours. The results reported are based on large numbers of observations on each of the 20 dogs employed.

[1] This work was made possible by a grant from the John and Mary R. Markle Foundation.

[2] Porter Fellow of the American Physiological Society.

Electrograms were picked up simultaneously by three pairs of contiguous electrodes recently described by Harris (2), and were recorded by three large Hindle galvanometers, properly aligned to obviate parallax. Time lines, cutting all curves, were recorded by spokes of a wheel actuated by a synchronous motor. The three pairs of contiguous electrodes were variously aligned and spaced in relation to each other and to the bipolar stimulating electrodes in different tests on the same heart, so that numerous points were studied. In some tests, the leading electrodes were arranged around the stimulating electrode as arcs of circles; at other times, linearly. An idea of the plans of arrangement in different tests is shown schematically in figure 1. One of these leads was usually placed as close to the stimulating electrodes as feasible without risk of damage to the galvanometer string. One of the advantages in using the contiguous electrodes for leads was that, by proper rotation, shocks as great as 40 M.A. could be

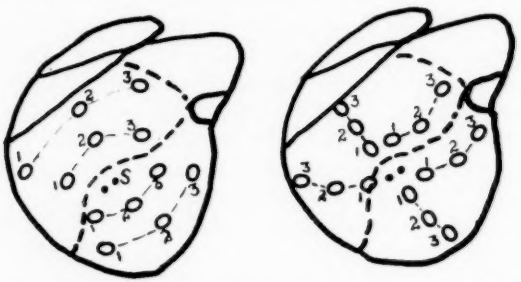

Fig. 1. Two diagrams indicating radial and linear placement of three pairs (*1, 2, 3*) of modified differential electrodes (contiguous electrodes) with respect to stimulating electrodes (S) in different experiments.

applied at a distance of 6 to 7 mm. The stimulating electrodes were also shifted in tests, the usual points selected being the apex, the left or right side of the ventricular septum, the base and pulmonary conus of the right ventricle.

In most of the observations the sinus node was clamped in order to slow the heart rate, and the heart was then driven by induction shocks applied to the right auricle. Every sixth beat, a brief D.C. shock 0.01–0.03 second in duration and ranging in strength from 5 to 40 M.A. was applied progressively earlier in a cycle through carefully plated Ag·AgCl electrodes, thus exploring the reactions at various moments of the whole cycle, systematically. For this purpose, the stimulator recently described by Wegria, Moe and Wiggers (5), which also alternated the direction of the current, was employed. It should be emphasized that shocks which evoke multiple systoles or fibrillation are several hundred-fold stronger than diastolic threshold shocks. Many special procedures, best described when pertinent topics are discussed, were also used.

Premature beats induced by late systolic stimuli. *Strong shocks* applied in late systole—determined either as mechanical systole from ventricular pressure curves, or as electrical systole of standard electrograms—evoke responses early in diastole (Wiggers and Wégria, 7). Such observations require explanation, first, because they seem to contradict the generally accepted doctrine that cardiac fractions are refractory up to the end of contraction, and secondly, because shocks which are strong enough evoke not one, but several beats early in diastole which may lead to fibrillation. In order to reconcile such findings with current knowledge, it has been suggested that this implies excitation of such myocardial fractions as have been repolarized or have stopped contracting somewhat earlier than is indicated by pressure curves or standard electrocardiograms (King, 3; Wiggers, 6). However, this meets with certain difficulties in interpretation recently discovered by Wégria, Moe and Wiggers (5). Therefore, experiments were carried out to determine whether smaller portions of ventricular myocardium respond when a shock is applied during electrical systole of localized fractions, i.e., during the Q-T segment of punctate electrograms recorded from spots adjacent to and more distant from the stimulating electrodes. As Harris (2) has recently emphasized, such electrograms represent the localized responses of a very small area of tissue under an electrode. When properly oriented in relation to the direction of normal excitation it consists of a sharp spike, the beginning of which is conveniently designated Q, and a final smaller deflection designated as T, much as in standard electrocardiograms. This Q-T interval unquestionably represents the duration of the electrical systole in very localized regions. When the direction of the impulse or rate of conduction change, the character of the deflection is also altered.

The character of these experiments is illustrated in figure 2, A, B, C, in which leads 1, 2 and 3 were taken from points similarly marked on the accompanying diagram. Brief D.C. shocks (30 M.A.) were applied as shown by the shock artefacts indicated by arrows. The three curves are taken from a long record of one experiment in which shocks were consecutively advanced from diastole to systole. They illustrate a single premature contraction after shocks delivered late in diastole (curve A), on the T wave (B), and definitely earlier than the T wave (C). The deflections are aberrant in form in leads 1 and 2, which were nearer the stimulating source, but retain their "spike characteristic" in lead 3, taken at a greater distance. The conclusion is warranted that points 1 and 2 received their excitation over abnormal, and point 1 ultimately over normal pathways.

Innumerable instances of systolic responses, such as are indicated in curve C, strongly suggest that nonrefractoriness during late systole cannot be explained on the basis of an early termination of contraction in certain areas of the myocardium. They are obviously not due to spread of current

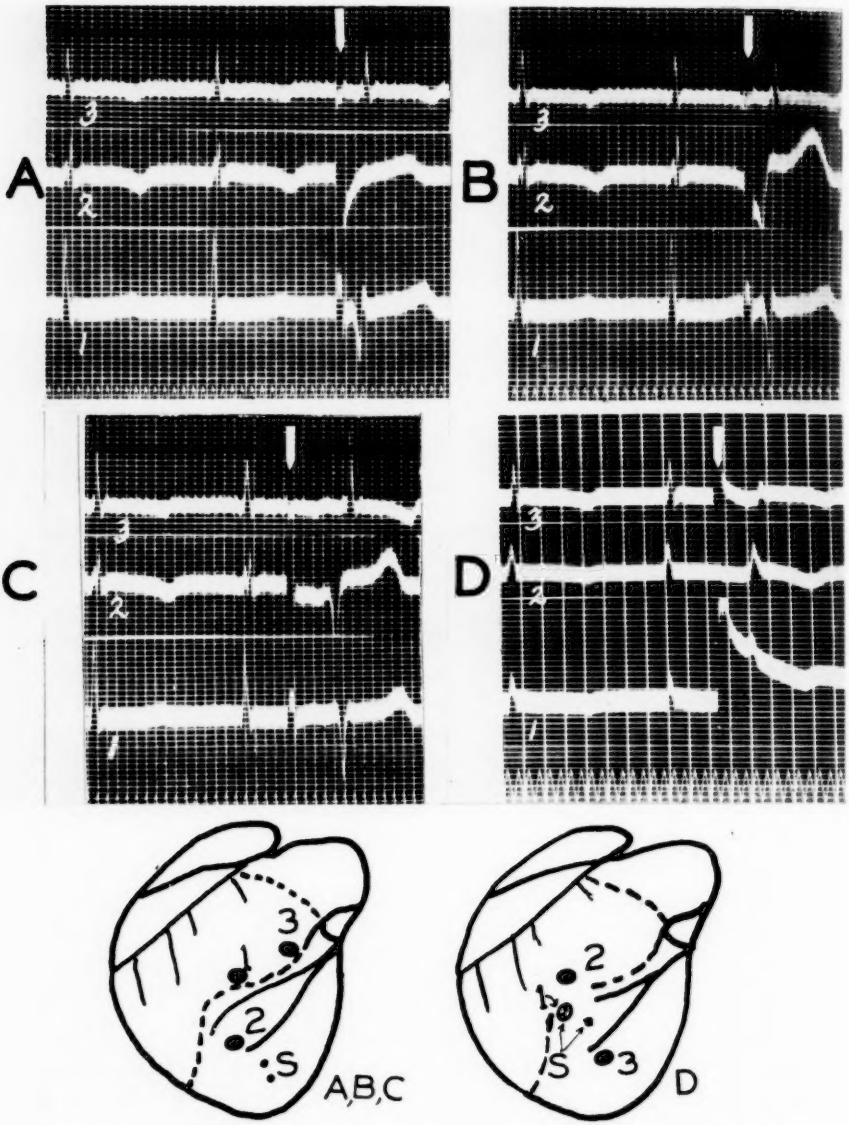

Fig. 2. Segments of records illustrating responses to D.C. shocks applied late in diastole (A), on the T wave (B), and preceding the T wave (C). Segment D shows a response in a lead taken from the stimulating electrode. Diagrams indicate location of punctate leads *1*, *2*, *3* and points of stimulation (S) in curves A, B, C and D respectively. Time, 0.02 sec. in A, B, C; 0.04 sec. in D.

to the auricle and subsequent re-excitation, as was again suggested by Woodbury (8) in the case of the turtle ventricle, for analysis of many curves invariably showed 1, that the order of initial response is related directly to the distance of leads from the stimulated points, and 2, that the contour of deflections changes in areas more adjacent to the source of stimulation, but generally remains the same in areas more remote from it.

The latency and interpunctal conduction times of single premature systoles. The latency of electrical responses occurring at different points on the ventricular surfaces after brief D.C. shocks applied at consecutive moments of the cardiac cycle as well as the interpunctate differences between points

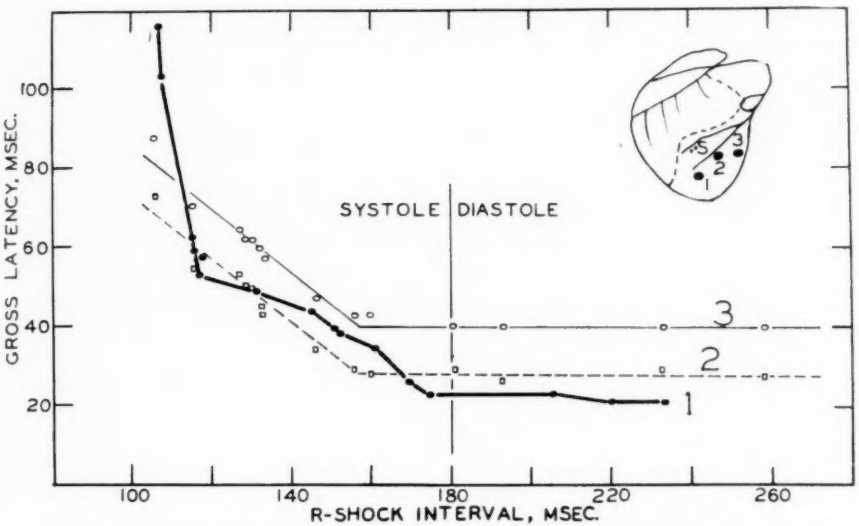

Fig. 3. Plot showing the constant latency of responses of three points (*1, 2, 3*) to shocks delivered during diastole, and the linear increase in gross latency as shocks are advanced into systole. Difference between lines gives interpunctal intervals. Abscissae, interval between preceding R deflection and shock.

in line with, and on the same side of, the stimulating electrodes have been measured.

Results showed that the latency of deflections resulting from shocks given during any portion of diastole and, in fact, as early as the summit of a T wave is always constant in any given lead. This does not agree with observations of Blair, Wedd and Young (1) on turtle strips in which conduction seemed to be affected by the diastolic interval. This gross latent period averages 12 to 15 msec. at points about 8 mm. from the stimulated locus and about 50 msec. at the most remote surface points on the anterior ventricular surface. Determination of latency at points near

the stimulating electrode is complicated by the shock artefact, except when low-voltage stimulation is used, in which case it can be detected at the top of the rectangular shock artefact. The interpunctal intervals likewise remain constant, regardless of the moment of diastole in which a stimulus is applied. This is illustrated by the plot of an experiment in figure 3.

When shocks are advanced so that they fall previous to the summit of T the situation changes. As indicated by the trend of lines in figure 3, the gross latency increases in linear fashion in most of the surface points as shocks are advanced in systole and may reach a magnitude as great as 100 msec. in very early shocks. In such instances, the pattern of the electrical deflections at any spot remains the same regardless of the moment at which the shock was applied. The only points which, in a few experiments, did not show such a methodical linear increase in latency were those on the apex; but in these the pattern of electrical deflections changed, suggesting involving of different conduction pathways.

Such progressive linear increase in the intervals of response might represent an apparent latency, really due to delayed conduction, or it may be a real latency of some sort. In favor of the former are many fundamental demonstrations indicating that any real latency of diastolic responses must be extremely short and perhaps nonexistent, differences being solely due to variations in conduction rates or conduction paths.

The following facts indicate, however, that delayed conduction does not suffice to explain why the latency increases linearly as strong systolic shocks are applied earlier and earlier in systole:

1. The interpunctal intervals, i.e., the time differences between points in line with and on the same side of the stimulated locus remain constant for responses to systolic and diastolic stimuli.

2. In specially designed experiments in which leads were taken directly from the stimulating electrodes, an appreciable lag still occurs. In order to protect the galvanometer string in such tests, a vacuum tube "shock absorber," which limited the pick-up of shock potential to safe ranges was introduced into the circuit.[3] Figure 2D shows such a record. The local response, of course reduced in amplitude, appears on the slow decline of the residual polarization curve. In this instance the delay was about 60 msec. and comparison with leads from other points shows a definite precedence.

As is evident in the sample record of figure 2C, while a strong localized stimulus applied during late systole is capable of evoking a response, such response does not occur previous to the end of the T wave. In other words, the interval from the previous normal spike to the shock, plus the latency, approximately equals the duration of a local electrical systole. These

[3] We are indebted to Dr. Harold Green for its construction and for assistance in its experimental use.

results must be squared with apparently demonstrated facts, viz., *a*, that heart muscle is refractory during the period of depolarization, supposedly extending from the spike to the T-wave; and *b*, that the energy of the stimulus is apparently "frozen," so to speak, until the early moments of diastole. It is conceivable that repolarization begins during the so called vulnerable period; indeed, Blair et al. (1) postulated occurrence of such a phenomenon in association with the waning of contraction previous to the T wave in strips of turtle ventricle. It is conceivable that heart muscle is excitable by very strong shocks and that an impulse is then conducted very slowly. However, this would not explain the retardation of local

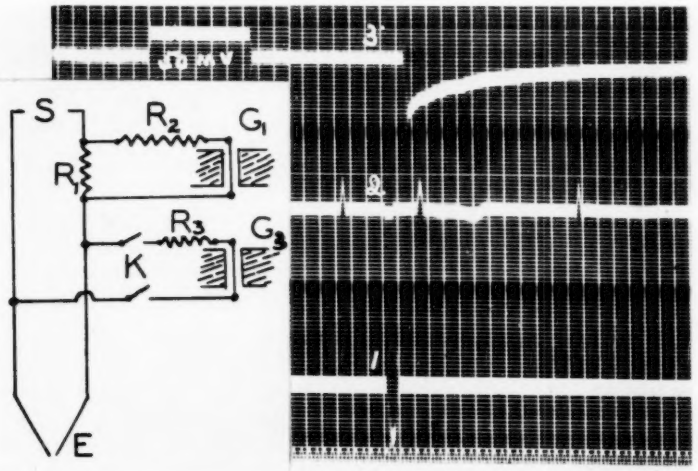

Fig. 4. Diagram of circuit used to determine magnitude and duration of polarization current resulting from strong brief D.C. shocks. Curves of lead 3 show calibration of string for 50 m.v. and by comparison that part of the polarization potential beginning about 20 msec. after the end of shock, indicated in lead 1. Time, 0.04 sec.

responses at the very point of stimulation, shown in figure 2D. Another possibility is that the tissue polarization resulting from a strong brief shock may be so great that it persists until early diastole and that the rate of decline of such polarization or its association with a sudden change in resistance during relaxation might make the waning polarization the real stimulus. In other words, while a strong shock is delivered during the refractory systole, its after-effects persisting into early diastole may be the real excitant.

In order to validate such a concept it is necessary to obtain evidence that polarization effects of such shocks last long enough and have sufficient intensity to be of excitatory value at the time of early diastole. Experi-

ments were done in which the magnitude of the polarization potential was recorded by a string galvanometer, temporarily disconnected during application of the shock. The arrangement used is shown in the diagram of figure 4. Galvanometer G_1, protected by a very high resistance, recorded the intensity of the shock. Galvanometer G_3, protected by an adequate series resistance (in this test 40,000 ohms), was disconnected briefly by an automatic circuit breaker and reconnected about 30 msec. after its application. The records, 3 and 1 of figure 4, show the electrical curves recorded by galvanometers G_3 and G_1; record 2 is a regular punctate lead by contiguous electrodes from a spot about 1 cm. distant. A calibration curve for 50 m.v. through the same resistance and electrodes is shown in an additional segment.

Analysis of such records showed that, at the time that the spike of lead 2 begins, curve 3 reveals a polarization potential of about 50 m.v. which gradually decreases to half-value in 0.08 second. By extrapolation of the exponential decline it was calculated that 20 msec. earlier, i.e., approximately at the time when the point under the stimulating electrode was stimulated—the polarization must have had a magnitude of about 100 m.v., which is probably sufficient to excite cardiac muscle during the early moments of the partially refractory phase. That this is a true polarization of tissues and not polarization of imperfect Ag-AgCl electrodes was proved by the fact that similar results were obtained after substitution of calomel-cell electrodes of special design.

Characteristics of sequential multiple deflections. Curves A, B and C of figure 5 show electrograms from two points more proximal to the source of stimulation and from one point more distal, as indicated on the accompanying diagram. They serve to illustrate reactions obtained in more comprehensive studies, too numerous to report by illustrations. They show the local potentials in various spots when a systolic D.C. shock (S) (0.02 sec.) evoked one deflection (curve A), four deflections followed by a pause and resumption of normal rhythm (curve B), and four deflections followed by true fibrillation (curve C).

The following facts stand out on careful inspection of these curves: 1. In general, the successive beats in leads 1 and 2 from points nearer the stimulated locus—even when these are on opposite ventricles—are aberrant in configuration, whereas those of lead 3 from a more distal region tend to retain their spike-like characteristic, which suggests that the latter are excited from a more nearly normal direction and probably over the regular Purkinje network.

2. The first of a multiple series of deflections (curves B and C) following a strong systolic shock is identical in form with that of a single response shown in curve A. This indicates that differences in the initial premature contraction cannot be concerned in initiation of fibrillation.

3. The four successive deflections (a, b, c, d) in either lead 2 or 3 of curves B or C differ essentially from each other in form; but corresponding beats (e.g., *b* in lead 2, curves B and C, or *b* in lead 3, curves B and C) re-

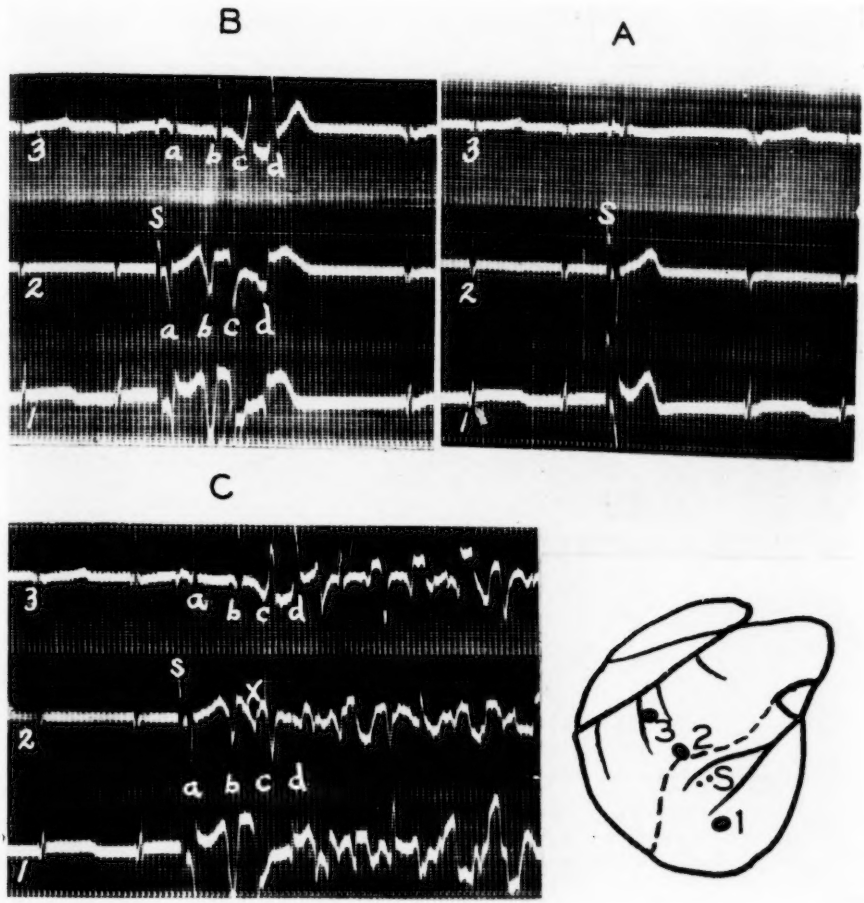

Fig 5. Three curves of punctate electrograms illustrating electrical responses to strong systolic shocks. A, a single deflection; B, multiple deflections followed by pause; C, multiple responses followed by fibrillation. Diagram shows orientation of leads *1, 2, 3* with respect to points of stimulation, *S*.

semble each other strikingly. This indicates that there is nothing peculiar in the nature of the second or third responses that could determine the temporary arrest or fibrillation which follows.

4. The second and third deflections in all leads are spaced at progressively closer intervals, but the shortening of periods is more marked in a proximal lead (2) than in the distal one (3). This will be discussed later.

5. The third deflection shows a similarity of form in leads 1 and 3 of curves B and C; but in lead 2 of curve C—nearest the locus of excitation—an inconspicuous earlier variation (x) appears which is not present in curve

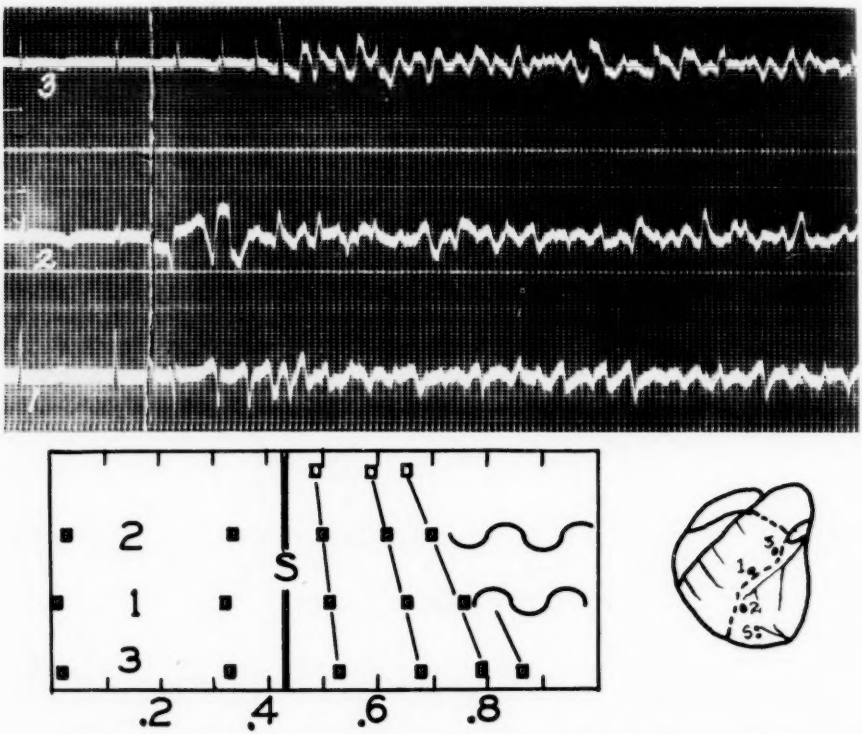

Fig. 6. Local electrograms from 3 points, as indicated in small diagram of heart, showing induction of fibrillation. Diagram illustrates interrelation of deflections and projection to a hypothetical center above. Time, 0.02 sec.

B. This indicates a disruption of rhythm at point 2 which appears to be related to a slight precedence of fibrillation at this point.

6. Despite the fact that a state of localized fibrillation starts at point 2 of curve C, the areas from which leads 1 and 3 were taken receive one additional interrelated excitation (d), just as in curve B, in which no fibrillation eventuated. The similarities in the forms and time relations of deflections *c* and *d* in leads 1 and 3 of records B and C clearly indicate that

in curve C, excitation of these areas—near and remote—are not yet due to or related to the fibrillating process started at lead 2.

The localized development of the fibrillating process. Such a localized fibrillation, which exists temporarily without involving other regions of the ventricular surface was found in a large number of instances, regard-

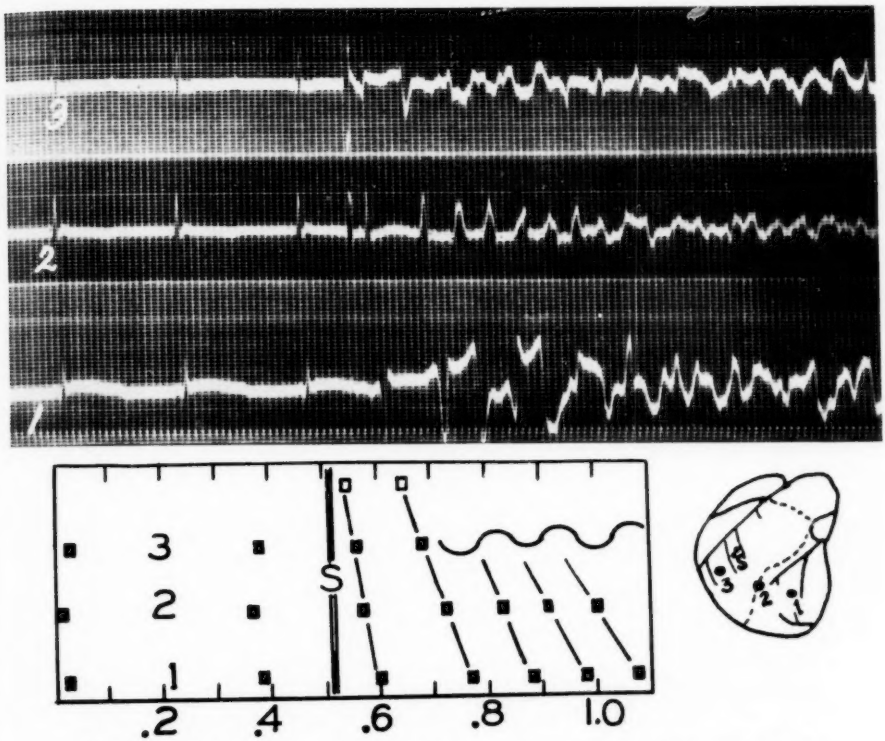

Fig. 7. Local electrograms from 3 points, as indicated in small diagram of heart, showing induction of fibrillation. Diagram illustrates interrelation of deflections and projection to a hypothetical center above. Discussion in text. Time, 0.02 sec.

less of the locus of stimulation. The fact is so important that a few additional curves are presented in confirmation.

Figure 6 shows simultaneous local electrograms from an experiment in which fibrillation was induced by a strong systolic shock near the apex. Leads 2, 1 and 3 are electrical variations recorded from points progressively more distant from the locus of stimulation, in the general direction of the interventricular septum, as indicated on the small diagram. A glance at the records indicates again that fibrillation occurs at the nearest

lead (lead 2), while at least three specific excitations at increasingly smaller intervals continue in leads 1 and 3. Such records show that there is no difference when more remote leads are taken over the septal surfaces. This has been extensively confirmed. Discussion of the plots of time relationships shown in figure 6 is advantageously deferred.

Figure 7 illustrates essentially the same phenomena following a strong systolic shock applied near lead 3 toward the base of the right ventricle. The disposition of leads was in the general direction of the superficial sinospiral bundle. We notice that the lead from area 3 nearest the point of stimulation breaks into incoördinate wavelets first. Curiously, the most distant lead 1 exhibits the greatest changes in form, while a septal point 2 lead more nearly retains the spike-like deflections and perhaps breaks into incoördinate wavelets a little later. This illustrates a type of experiment in which it appears probable that more remote areas do not continue to be excited from the initial focus, but rather from a slowly advancing fibrillating front.

Numerous experiments in which the three leads were placed along surface bundles failed to show, as in this case, that the fibrillating process necessarily spreads in the direction of surface bundles. This is not surprising in view of the complex intertwining of conduction pathways of normal and Purkinje fibers which exists in the myocardium. Such observations disprove the possibility that development of fibrillation is contingent on passage of repeated long reëntrant waves over both ventricles by common fasciculi of fibers.

Cause of the multiple accelerating beats. The intimate relation of the multiple accelerating beats to fibrillation demands the most searching inquiry possible as to their cause and the part they play in the breakdown of an orderly excitation in an initially restricted area. Two causes can be thought of to account for the preliminary undulatory movements of fibrillation which are reflected in the localized electrograms we have been describing: (1) With the exception of the first, which is clearly a premature systole, they may represent a series of reëntrant impulses, or (2), they may be due to repetitive discharges from an area around the excited points.

The first suggestion was rather favored by one of us (6), partly on observations of the surface movements in films; partly on the basis of the diminishing spacing and continually changing forms of standard electrocardiograms. If conduction rates and refractory periods of cardiac muscle established by previous investigators are accepted, such a circuit would have to be a relatively large one, and some portion of the myocardium would have to be excited every moment after the first response.

The best method for detecting such circuits would be to take numerous surface leads simultaneously during the occurrence of the second and third beats. However, evidence should be obtainable in repeated fibrillations

showing the same general character of waves in which records are taken by the only three leads available to us. Such experiments have been carried out and surface plots of the results made. An example is shown in figure 8 in which the incidence of surface negativity after a single diastolic response and the first three cycles of multiple responses to a strong systolic shock are plotted separately. Lines have been drawn at intervals representing 10 msec. differences in time of excitation. Such *isochrons* in every case have a roughly concentric configuration about a stimulated locus. *They must not be regarded as a chart of the actual spread of the excitatory wave front but rather as a chart of the sequence of surface activation, perhaps over deeper routes.*

An inspection of the four diagrams reveals that the pattern of spread of the first response following a strong systolic shock does not differ essentially from that of a single response to a diastolic shock. In both, the order of excitation is radial, in all directions from the stimulated area. The other diagrams indicate that successive responses of a train likewise radiate from the stimulated locus, often as far as the fourth deflection. This favors the view that all originate at or near the site of stimulation.

The distances between the isochrons of figure 8 indicate that, after allowing for the greater gross latency, the first response following a systolic shock reaches identical surface points at approximately the same time as diastolic shocks (cf. drawings A and B). Since the configuration of complexes is also essentially the same in the two cases, it may be assumed that the impulses traveled over the same routes and that, therefore, the conduction time is approximately the same. Since the route of travel is unknown, estimates of conduction rates are, of course, hazardous. In the case of the 2nd or 3rd beat of multiple complexes, shown in C and D of figure 8, the impulses arrive at the surface more and more slowly. This may denote an actual decrease in rate of propagation, which is in accord with knowledge that the conduction rate decreases with shortening pauses; but such a conclusion cannot be validated by our observations owing to the significant changes in contour of successive complexes analyzed in figure 5, which indicate that the different pathways were probably followed. However, regardless of the cause of the progressive delay in activation of surface points, its existence cannot be disputed.

Such plots are difficult to reconcile with the idea that the 2nd or 3rd initial complexes are due to reëntry of impulses. In many records of such beats, followed by fibrillation or not, we have never seen sequential surface changes that would fit the idea of long circuits of reëntry. The only conceivable way in which reëntrant waves could give rise to such isochrons would be to postulate that the reëntrant wave returned each time by way of deeper tissues, thus preventing its detection by surface leads. This possibility is much reduced by experiments in which internal leads, taken ap-

proximately opposite to the external ones, showed no such time lag as would be demanded if the interior represented the return path.

If the 2nd and 3rd beats represent a reëntry, a unilateral block must be produced by the shock and we would expect to find spots of delayed excitation to one side of the stimulated region in our numerous curves, at least occasionally. None have ever been found. Furthermore, according to the reëntry hypothesis, some portion of the myocardium must be excited

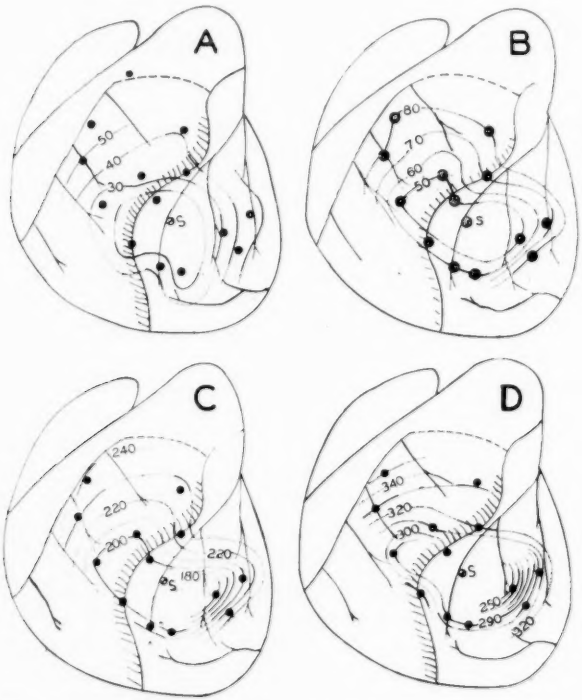

Fig. 8. Diagram showing isochrons of responses to a single response, A, to a diastolic shock, and the first three cycles (B, C, D) of multiple responses following a strong systolic shock.

at all times. Our numerous observations on the ventral surface and anterior wall always had a gap in which no point was excited. Thus the latest time following excitation at which any portion of the anterior surface was excited in figure 8B was 80 msec., and the earliest moment after excitation that the surface again became negative was 180 msec. (fig. 8C). It is difficult to believe that excited spots could have all been missed during the 100 msec. which intervened. Similarly, a gap of quiescence equal to 80

msec. intervenes between the plots of figure 8C and D. Hence, our conclusion that no portion of the heart was excited during this interval. Finally, if we retreat to the view that a small localized zigzag system develops in the vicinity of the locus of stimulation, it must have been less than the distance to the nearest electrode. This would require an inconceivable degree of slowing of conduction.

Since the analysis thus far indicates *a*, that the time of activation of concentric points around the stimulated locus increases progressively in succeeding beats; *b*, that no clear evidence of circus rings exists in the early deflections which precede fibrillation, and *c*, that the gross latency of response of concentric fields for each cycle increases systematically away from the point of stimulation, it appears improbable that reëntry of impulses can explain the 1st, 2nd and, occasionally, the 3rd and 4th beats which lead to fibrillation. It rather favors the view that they are due to repetitive firing of an area near the point of stimulation.

In the two diagrams of figures 6 and 7 respectively a crude attempt is made to illustrate the manner in which this might operate up to the time when fibrillation develops at each more distant spot. The interrelationships of spikes in the three leads while the myocardium is normally excited is indicated by the first two sets of small black squares. The time of the shock is denoted by S; and for each lead the time interval at which excitation manifests itself is indicated by another small black square. Lead 2 is nearest the locus; lead 3, farthest away. A glance at such a graph—even better than the curve itself—shows that the responses are spaced more closely at the adjacent than at the most distant point. Since the lead from the nearest point was 8–10 mm. from the stimulated locus, the latter must have discharged still more rapidly. This can be pictured by drawing slanting lines through the plotted squares and extending them to some arbitrary line indicated by white squares at the top of the graph. They represent the still more rapid tempo of the discharging area. The sloping lines, themselves, denote the progressive delay of impulses on surface points. Such a scheme helps to emphasize the idea that the beats preceding fibrillation are due to repetitive firing of impulses from a center, each one of which is transmitted more slowly to various muscle fractions. It also visualizes the common precedence of fibrillation at proximal points, while more distant ones continue to receive the retarded impulses. Admittedly, such a state of localized fibrillation is very temporary, but its brief existence is nevertheless important in analyzing the mechanism by which fibrillation starts.

The effects of accelerated induction shocks. One fact chiefly requires to be harmonized with the concept that several repeated impulses are discharged from a center, viz., the changing configuration of the successive deflections. They certainly suggest that the impulses reach the con-

tiguous electrodes from different directions, and hence that conduction disturbances are present at the same time. These disturbances may be

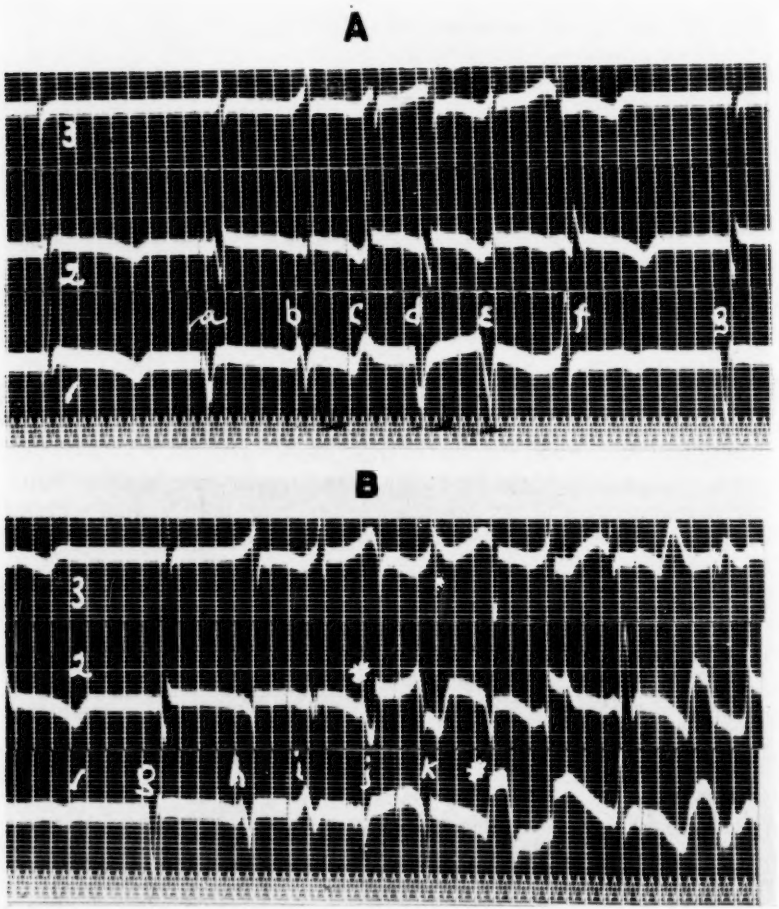

Fig. 9. Curves of preliminary electrographic deflections and fibrillation produced by an accelerating series of threshold induction shocks. A, no fibrillation, only aberrant deflections (*a*–*e*) changing from cycle to cycle; B, similar deflections (*h*–*k*) degenerating to re-entrant beats at *. Fibrillation precedes in lead 2. Time, 0.04 sec.

physiological consequences of the rapid repetitive impulses; or they may be due to a physical influence of the D.C. shock *per se*.

Furthermore, if our suspicion is correct that fibrillation develops as a

result of 2 to 4 progressively retarded impulses fired from a common center, it should be possible to reduplicate similar initial deflections and to produce fibrillation by applying to an area an artificial series of stimuli, approximately of threshold value.

Accordingly, experiments were designed in which five weak break induction shocks (makes short-circuited) were applied at diminishing intervals during a long diastole. This was accomplished by using as key a disc with five cams spaced progressively closer, each one operating a set of keys. The speed of the camshaft determined the actual intervals.

Experiments showed that a spacing of the accelerating stimuli resembling the rhythm produced by one strong D.C. shock could be achieved by trials. When such shocks are applied too far apart or come too close together fibrillation is not invoked; when properly spaced it follows regularly.

Figure 9 shows two illustrations. In A, a set of five threshold shocks is followed by a series of deflections in lead 1, nearest the stimulated area, each of which has a different configuration. This demonstrates that the conduction disturbances are associated with the rapidly repeated excitations and are not due to a direct effect of the current *per se*. In this record, the series marked *a*, *b*, *c*, *d*, *e* is followed by a spontaneous beat (f). Before a natural rhythm could be restored another artificial series of threshold break shocks were applied. The continuation of the record—in which deflection (g) is repeated—is shown as curve *B*. Four bizarre deflections (h–k) occur which are followed by fibrillation, indicated in leads 1 and 2 by an asterisk (*). The broad abnormal character of deflections due to reëntry is well shown.

Discussion. *A theory of the initiation of ventricular fibrillation.* We accept the demonstration of Garrey (4) that the incoördinate state of contraction to which the term "fibrillation" should be restricted is due to circus excitation. This is universally preceded, however, by a run of tachysystolic beats which hold the secret as to the mechanism of onset of fibrillation. The observation—important in itself—that a brief strong shock must coincide with the vulnerable period of late systole or very early diastole in order to produce such tachysystolic beats and fibrillation is not itself an answer to the question as to why it does so. We must know how a stimulus falling during a supposedly refractory phase can stimulate at all, and we must understand the physiological process it sets up which quickly makes reëntry and circulation of impulses possible.

We have presented evidence that a late systolic shock is really effective because it creates a tissue polarization of sufficient strength and duration to excite at the very first moments of the relatively refractory period. Moreover, it appears to start a rhythmic center from which several impulses are discharged at an accelerating rate, the limit of the interval being about 80 msec. These successive stimuli arrive at more remote regions, later

and later. The progressive decrease in refractory periods associated with the accelerating rate of responses, combined with delay in conduction, furnish ideal conditions for reëntry of impulses, *but only after the second, third or fourth truly premature beats have run their course.* Since myocardial fractions nearest the stimulated locus are excited at shorter intervals than more distal ones, their refractory period decreases most, and reëntry occurs there first. This explains why localized areas of fibrillation exist temporarily near the site of stimulation, while more remote ones temporarily receive periodic coördinated excitations.

Without prejudice to the view that primary alterations in conduction may account for the onset of ventricular fibrillation under other circumstances, our results do not support the view that immediate changes in the duration of the refractory period or of conduction time are responsible for fibrillation produced by a brief strong D.C. shock. On the contrary, such changes are *physiological consequences* of a rapid accelerating discharge of impulses from a center near the stimulus. The bearing of these findings on the interpretation of "spontaneous fibrillation," e.g., after coronary occlusion becomes evident on reflection.

A generalized state of fibrillation quickly develops as a result of either of two mechanisms: 1, numerous areas may start independent fibrillating centers in rapid sequence by the same mechanism and then merge, or 2, the fibrillation spreads from the initially fibrillating area to adjacent ones by reëxciting fractions as soon as they have passed out of their refractory phase. Our analysis of many records indicates that the former is relatively uncommon; the latter, usual.

SUMMARY

The mechanism by which ventricular fibrillation develops as a result of a strong, brief D.C. shock delivered during the vulnerable period of the ventricular cycle was studied electrographically. Three pairs of contiguous electrodes operating on the principle of Garten differential electrodes were variously oriented on the ventricular surface with respect to the site of stimulation in different tests on the same heart. Since the ventricles were repeatedly revived by the countershock method of Hooker, the ventricular surface was sampled reasonably well by changing the placement of electrodes.

The following results and conclusions are discussed:

1. A moderately strong, brief shock, applied considerably before the T wave of an electrogram recorded at, or near, the point of stimulation causes one response shortly after the T wave. Tests are presented which indicate that the response is probably not due to an actual systolic excitation but to the creation in tissues of a decrementing polarization potential which is sufficient in duration and intensity to excite early in the next relatively

refractory phase. In this way, the response of the ventricle to a systolic shock can be harmonized with the existence of a refractory state in muscle fractions. It also explains why the latency of responses at definite points on the cardiac surface remains constant for shocks applied at any moment of diastole, but increases linearly as they are applied more and more in advance of the T wave of a local electrogram. No evidence has been found in the dog's heart that changes in conduction are involved in such increasing latency; the interpunctal differences between excitation lines remain the same, regardless of when a stimulus is introduced.

2. A very strong shock applied to a discrete mass of ventricular muscle during the Q-T interval of an adjacent electrogram evokes a series of deflections in all electrograms led from the cardiac surface. This may be followed by a pause and resumption of normal rhythm or by fibrillation. When the latter occurs, it develops in the nearest surface lead slightly before it does in the others.

3. The series of discrete deflections recur at progressively decreasing intervals in the most proximal lead and the excitation times more distant in relation to proximal points increase progressively during the series of responses.

4. The order of excitation on the surface and interior of the ventricle during the second, third and fourth responses and the existence of a period between beats when no area is excited do not support the view that the initial beats are due to reëntry of impulses. On the contrary, the concentric arrangement of isochrons calculated from actual records strongly suggests repetitive emission of several impulses from the stimulated area, temporarily made automatic by the shock.

5. Deflections similar in form and sequence in near and far areas of the heart could be caused artificially by applying a series of weak threshold break induction shocks at slightly diminishing intervals. If given in proper sequence, these also cause fibrillation.

6. The conclusion is drawn that the reëntry of impulses with which fibrillation following localized application of a strong, brief D.C. shock starts, is due to the progressive decrease in refractory period combined with a progressive increase in conduction time. This starts in regions near the site of stimulation and occurs as a result of the repetitive accelerating discharges rather than as an effect of the current *per se*. In short, while repetitive discharges from a center or centers are not required to sustain fibrillation, they are essential to its initiation after a strong electrical shock.

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THE RELATIONSHIP OF RENAL BLOOD PRESSURE AND BLOOD FLOW TO THE PRODUCTION OF EXPERIMENTAL HYPERTENSION[1]

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It appears well established (1, 2, 3) that radical alterations in the normal hemodynamics of the kidney may produce experimental hypertension. It has been demonstrated (4, 5) that in all probability such an altered kidney produces a pressor substance de novo or an increase in the amount of pressor substance normally secreted into the blood stream. As a matter of fact, a substance has been found (6) in the venous blood leaving the partially ischemic kidney of the dog, which effected a marked and rapid pressor response when given to another dog. This same substance may be neutralized, however, by the intact, functioning kidney. In its chemical and physiological properties, this particular substance was thought to be similar in certain respects to both purified renin and angiotonin (7), but not identical to either.

Despite the above observations, the initiating factor in the production or increased production of this renal pressor substance has not been clearly demonstrated. Constriction of the renal artery by a Goldblatt clamp can conceivably effect only two hemodynamic changes in the kidney, namely, 1, a reduction in the renal blood flow, or 2, a reduction in the renal blood pressure, including the pulse pressure, distal to the clamp. In the majority of clamp applications, probably both of these changes occur. Recently, however, there have been several observations recorded (8, 9, 10) which suggest that the kidney in experimental hypertension need not be ischemic. Furthermore, renal blood flow determinations performed upon human patients suffering with essential hypertension (11, 12) demonstrate that renal ischemia is not an invariable finding in this syndrome. Even in many of the cases in which a renal ischemia is present, it is frequently of so slight a degree that a legitimate doubt is justified as to whether the ischemia was the causative factor in the maintenance of the hypertension. Finally,

[1] We are indebted to the Winthrop Chemical Company for their kindness in supplying us with generous quantities of diodrast.

it may be mentioned that the detection of renin in the blood leaving the perfused kidney (13) is dependent upon a reduction in the pulse pressure rather than in the rate of blood flow to this same kidney.

In the present communication, evidence is presented which indicates that renal ischemia is neither the initiating, the causative, nor the maintaining factor in the production of experimental hypertension.

I. The renal circulation before, during, and after a constriction of the aorta above the aortic orifices of both renal arteries (acute). It has been recognized that the renal blood flow may remain unchanged despite alterations in the arterial pressure (14, 15, 16), but the intra-renal causes for this are not definitely known. Accordingly, the renal blood flow, the glomerular filtration rate, and the filtration fraction were observed before, during, and after a constriction of the aorta above the orifices of both renal arteries.

Methods. Six normal dogs (series A) were anesthetized with pentobarbital sodium. A left lumbar incision was made, the peritoneum incised, and the aorta above both renal arteries exposed and encircled by a loose tape ligature, the two ends of which were conducted to the outside through a brass tube 2.5 mm. in diameter. The wound was then closed. During this manipulation the kidneys were not disturbed. The left femoral artery was cannulated and connected to a mercury manometer, allowing continuous blood pressure recording. The left femoral vein was connected to an infusion flask, and for an hour following the application of the aortic ligature, the animal slowly received an infusion of normal saline solution (150 cc.).

After preliminary intravenous administration of diodrast (0.5 per cent) and inulin (1.66 per cent) in saline solution for 30 minutes, the renal blood flow, the inulin clearance, the mean femoral blood pressure and in some cases the femoral pulse-pressure were obtained during a 30 minute control period. The ligature was then tightened until a decrease of 30 to 40 mm. Hg was observed in the femoral artery pressure and this degree of reduction was maintained for 40 minutes. During this constriction period, the above determinations were repeated after an initial delay of 10 minutes. The third and final determinations were made in a 30 minute period following the release of the aortic constriction.

In five other dogs (series B) exactly the same procedure was followed except that the aortic ligature was tightened until a fall of 40 to 60 mm. Hg in the mean femoral artery pressure occurred.

For the determination of the renal blood flow and the inulin clearance of each 30 minute period, three catheterized urine collections (each of 10 min. duration) and two blood samples were taken. These were then analyzed separately for iodine (according to the method of White, 17) and for inulin (according to the method of Alving, 18). The mean of these three determinations was taken as the average period value. The diodrast

plasma clearance in cubic centimeters per minute plus the addition of the volume of red blood cells (determined by hematocrit) was taken as the renal blood flow. The factor advocated by White (19) was not used in the calculation of the renal blood flow because our interest was primarily in comparative values. The filtration fraction was obtained as the inulin plasma clearance/diodrast plasma clearance.

The aortic and renal artery hemodynamics below the constriction were studied by blood pressure determinations obtained from the femoral artery. It was thought that, although there might be slight differences in the actual pressure values in these three sites, the determination of the pressure values, particularly changes, in the femoral artery afforded a reasonably accurate picture of the intra-aortic and intra-renal artery hemodynamics. Accordingly, the aortic and renal artery pressure values cited in this communication were those actually found in the femoral artery. The phasic blood pressure determinations were obtained with the Hamilton apparatus.

At the end of each experiment the animal was autopsied, the position of the aortic ligature relative to the renal artery orifices was checked, and the kidneys weighed.

Results. In the dogs of series A (see table 1-A) it was observed that although a reduction in the aortic mean pressure of 30 to 40 mm. Hg below the constriction led to a marked decrease in the pulse pressure at the expense of the systolic phase of the blood pressure, there was no significant change in the renal blood flow as measured by the diodrast clearance. In three of the six dogs there was a slight increase in flow, in the remaining three a slight decrease with an average reduction in flow of 1.5 per cent, which was within the experimental error. In all dogs a reduction in the glomerular filtration, as measured by the inulin clearance, was observed. The average decrease was 20 per cent. This fall in glomerular filtration rate resulted in a concomitant fall in the filtration fraction (see table 1-A).

The maintenance of a normal renal blood flow in these dogs during aortic constriction, despite a moderate reduction in the mean pressure and a severe decrease in the pulse pressure of the aorta and renal artery, indicated that renal vascular dilatation must have occurred. The average decrease of 20 per cent in the glomerular filtration rate indicates that there must have been a reduction in the filtration pressure within the glomerulus. The exact site of this renal vascular dilatation could not be definitely determined although the decrease in glomerular filtration despite a maintenance of a normal blood flow suggested that a dilatation chiefly involving the glomerular efferent arterioles had occurred. It is likely that the afferent arterioles also dilated, but certainly to a lesser degree. In preliminary experiments it was found that if constriction were maintained over 30 minutes a decrease in the renal blood flow uniformly occurred. Even during the 30 minute constriction period, the last 10 minute determination

of the renal blood flow was usually less than that obtained during the first 10 minutes.

After the release of the aortic constriction the mean renal artery and pulse pressure in each dog returned to a value as high as or higher than the pre-constriction value. The inulin clearance also returned to about the

TABLE 1

The renal circulation before, during, and after a constriction in the aorta above both renal arteries

EXPERIMENT NUMBER	BEFORE CONSTRICTION					DURING CONSTRICTION					AFTER RELEASE OF CONSTRICTION				
	Mean femoral pressure	Femoral pulse pressure*	Renal blood flow†	Renal inulin clearance‡	Filtration fraction	Mean femoral pressure	Femoral pulse pressure	Renal blood flow	Renal inulin clearance	Filtration fraction	Mean femoral pressure	Femoral pulse pressure	Renal blood flow	Renal inulin clearance	Filtration fraction
A. Constriction causing reduction of 30–40 mm. Hg in femoral pressure															
	mm. Hg	*mm. Hg*			*per cent*	*mm. Hg*				*per cent*	*mm. Hg*	*mm. Hg*			*per cent*
3	115		2.74	0.57	38.0	78		2.45	0.47	32.2	120		2.38	0.60	46.9
4	110		3.69	0.74	19.9	85		4.56	0.67	21.0	132		3.06	0.74	31.5
8	120	50	3.70	0.74	30.9	85	15	4.53	0.59	23.3	145	75	3.07	0.74	37.8
9	122	75	3.32	0.52	25.7	85	15	3.16	0.48	24.5	135	70	2.93	0.42	22.9
13a	100	40	3.93	0.74	14.7	62	20	1.49	0.46	24.2	100	40	2.38	0.71	23.2
14a	140		4.08	0.56	24.4	100		5.00	0.45	15.9	140		3.22	0.41	20.8
Average	118	55	3.58	0.65	25.6	82.5	16.66	3.53	0.52	23.5	128	67	2.84	0.60	30.5
B. Constriction causing reduction of 40–60 mm. Hg in femoral pressure															
2	110		2.60	0.81	31.4	65		2.51	0.66	27.7	105		1.87	0.65	35.0
5	150	60	2.92	0.60	28.0	100	10	2.98	0.57	27.6	135	55	1.85	0.59	56.2
6	155		4.30	0.78	28.9	100		2.94	0.61	32.1	160		2.69	0.78	45.4
7	135	65	3.02	0.63	34.2	82	10	2.78	0.53	31.2	135	60	3.38	0.89	43.9
10	140		3.73	0.57	22.6	86		2.94	0.44	22.1	110		2.73	0.48	26.5
Average	138	62.5	3.31	0.68	29.0	87	10	2.83	0.56	27.9	129	57.5	2.50	0.68	41.4

* Obtained with the Hamilton Blood Pressure Apparatus.

† Calculated as the diodrast clearance in cubic centimeters per minute per gram of kidney divided by the hematocrit plasma volume percentage.

‡ Calculated as the inulin clearance in cubic centimeters per minute per gram of kidney tissue.

pre-constriction clearance, but the renal blood flow sharply decreased (average reduction, 20 per cent). This comparative ischemia, despite the normal arterial pressure and glomerular filtration rate, strongly suggested that an increase in the tonus of the glomerular efferent arteriole had occurred. For it was difficult to observe any other change in the kidney

circulation which had taken place that could have reduced the rate of flow and not the rate of glomerular filtration. This ischemia, however, was usually temporary and disappeared at the end of an hour.

In the dogs of series B, it was found that when the mean aortic pressure was reduced 40 to 60 mm. Hg, there was not only a decrease in the pulse pressure (average reduction, 84 per cent), and in the inulin clearance (average reduction, 16.5 per cent), but also in the blood flow (average reduction, 14.5 per cent). The filtration fraction likewise showed a slight decrease (average reduction, 4 per cent). Aortic constriction of this severity, then, decreased not only the pulse pressure and the glomerular filtration rate, but also the renal blood flow. As already observed in the dogs of series A, the release of the constriction was followed by a return of the inulin clearance and the pressure to about pre-constriction levels. The renal blood flow, however, decreased even more (average reduction, 24 per cent) after release of the constriction than had been observed in the dogs of series A. The filtration fraction (average filtration fraction, 41.4 per cent) thus became even higher and represented a 43 per cent increase over that found during the control period. Here again, the return of all factors studied to the pre-constriction level with the exception of the renal blood flow pointed to the probability of the occurrence of increased glomerular efferent arteriolar tonus.

II. The renal circulation before, during, and after a constriction of the aorta above the orifice of the left, but below the orifice of the right renal artery (acute). Methods. Six dogs were studied. Exactly the same procedure was carried out as previously described in dogs of series B (40–60 mm. Hg reduction in mean aortic pressure), except that the ligature was placed around the aorta above the left renal, but below the right renal artery aortic orifice. By this procedure, then, one kidney with normal hemodynamics was present in a dog whose remaining kidney was subjected to arterial pressure changes. Preliminary determinations of carotid artery pressures prior to and following the aortic constriction revealed no consistent changes in aortic pressure above the constriction.

Results. As table 2 clearly demonstrates, although the effects of aortic constriction upon one kidney gave total renal changes roughly parallel to those found previously in dogs of series B, except for a lesser degree of renal ischemia, a return of the renal blood flow to normal, together with an actual decrease in the inulin clearance and filtration fraction, occurred after the release of the constriction. These last observations suggest that the increased filtration fractions observed in dogs of series A and B were not due to neurogenic or mechanical factors, but were due in all probability to the elaboration of a humoral substance, apparently formed during the period of low arterial and pulse pressure in the *absence of renal ischemia* (see table 1-A). In this last series of dogs, this substance was probably

neutralized by the normal kidney present for, as has been mentioned (6), the normal kidney appears to neutralize quickly the effect of the pressor substance present in the blood leaving a kidney whose artery has been severely clamped.

III. The renal circulation and the systemic blood pressure, before, during, and after a reduction in the aortic blood pressure (acute and chronic). The above observations strongly suggested that a kidney subjected to a moderate reduction in its mean pressure and a severe reduction in its pulse pressure nevertheless could maintain a normal renal blood flow for a short period of time and that during this period it apparently produces a substance capable of causing glomerular efferent arteriolar spasm. However, no immediate systemic hypertension was observed in any experiment. It was thought advisable therefore to determine whether this substance apparently formed by a non-ischemic kidney under reduced pressure (mean and pulse) would later produce a chronic hypertension.

TABLE 2

The renal circulation before, during, and after a constriction in the aorta above the left, but below the right renal artery

EXPERIMENT NUMBER	BEFORE CONSTRICTION					DURING CONSTRICTION					AFTER RELEASE OF CONSTRICTION				
	Mean femoral pressure	Femoral pulse pressure	Renal blood flow*	Renal inulin clearance†	Filtration fraction	Mean femoral pressure	Femoral pulse pressure	Renal blood flow	Renal inulin clearance	Filtration fraction	Mean femoral pressure	Femoral pulse pressure	Renal blood flow	Renal inulin clearance	Filtration fraction
	mm. Hg	*mm. Hg*			*per cent*	*mm. Hg*	*mm. Hg*			*per cent*	*mm. Hg*	*mm. Hg*			*per cent*
1b	125		3.94	0.77	34.0	82		3.72	0.57	23.8	110		2.8	0.44	27.2
2b	135		3.36	0.69	29.9	85		3.62	0.73	28.2	135		4.0	0.73	26.6
3b	125	55	3.66	0.58	23.8	82	15	3.72	0.36	14.5	132	65	3.93	0.61	23.3
4b	125		3.29	0.59	27.0	85		2.55	0.42	25.0	120		4.03	0.59	23.3
5b	125	35	4.7	0.62	34.4	85	8	3.39	0.49	35.0	125	37	4.0	0.42	26.7
6b	130	35	4.13	0.54	22.0	85	10	2.96	0.42	24.1	85	30	3.5	0.36	17.4
Average	128	42	3.85	0.63	28.5	84	11	3.32	0.49	25.1	118	44	3.71	0.53	23.9

* Calculated as the diodrast clearance in cubic centimeters per minute per gram of kidney divided by the hematocrit plasma volume percentage.

† Calculated as the inulin clearance in cubic centimeters per minute per gram of kidney tissue.

Methods. Five uninephrectomized dogs, previously trained for blood pressure determinations, were used. Uninephrectomized dogs were used in order to avoid the extensive surgical manipulation associated with the application of the aortic clamp above two renal arteries. A preliminary renal denervation was also performed on one of the five dogs (4-c).

After stable blood pressure levels had been reached, the dogs were anesthetized with pentobarbital sodium, the aorta was exposed as before and encircled by a silver clamp, although no compression of the aorta was effected at this time. The clamp itself was similar to the Goldblatt designed clamp, except considerably larger and equipped with a rectangular screw head, over which a tightly fitting rectangular tube extending to the outside could be fitted. The clamp and tube having been connected, the field was quickly closed, and the tube allowed to project from the wound. After one hour the renal blood flow, inulin clearance and blood pressure (femoral) were determined over a 30 minute period as control values under anesthesia. A needle attached to a mercury manometer

TABLE 3

The effect of a constriction of the aorta upon the femoral blood pressure and the renal circulation (acute and chronic)

EXPERIMENT NUMBER	CONTROL VALUES					ACUTE EFFECT OF COARCTATION				CHRONIC EFFECT OF COARCTATION				
	Control blood pressure*	Control blood pressure under anesthesia	Renal blood flow†	Renal inulin clearance‡	Filtration fraction	Femoral blood pressure	Renal blood flow	Renal inulin clearance	Filtration fraction	Maximal femoral blood pressure after constriction	Duration of hypertension	Renal blood flow (72 hrs. post aortic constriction)	Renal inulin clearance (72 hrs. post aortic constriction)	Filtration fraction (72 hrs. post aortic constriction)
	mm. Hg	*mm. Hg*			*per cent*	*mm. Hg*			*per cent*	*mm. Hg*	*days*			*per cent*
1c	135/100	140/110	354	94.2	36.3	110/100	326	63.5	27.6	155/130	7			
2c	125/85	130/90	205	42.5	36.3	105/95	247	43.5	32.1	138/118	14			
3c	120/90	130/90	236	54.0	34.2	80/75	205	45.3	33.1	150/140	14	210	51.5	36.8
4c§	140/85	140/95	164	28.6	26.7	105/95	158.5	22.2	21.4	130/120	12	173	41.5	37.0
5c	130/80	140/95	221	52.4	32.4	105/95	323.0	38.2	16.9	105/95	0	312	72.0	33.9
Average	130/90	136/96	236	54.3	33.1	101/92	251.9	42.5	26.2	135/121				

* All blood pressures obtained from right femoral artery with Hamilton Blood Pressure Apparatus.

† Calculated as the diodrast clearance in cubic centimeters per minute per square meter of surface area divided by the hematocrit plasma volume percentage.

‡ Calculated as the inulin clearance in cubic centimeters per minute per square meter of surface area.

§ Kidney previously denervated.

was inserted into the femoral artery and the tube connected to the aortic clamp was turned until there was a reduction of 20 to 30 mm. Hg in the mean femoral pressure. After 10 minutes a second renal blood flow and inulin clearance determination was performed over a 30 minute interval. The tube was then disengaged from the aortic clamp by means of a plunger insertion, allowing the clamp to remain encircling and compressing the aorta. The femoral blood pressure of the five animals was followed daily, and in three of the five dogs a repeat renal blood flow and inulin clearance determination was performed 72 hours after aortic constriction. The renal blood flow and inulin clearance values, adjusted to one square meter of surface area, are given in table 3.

Results. As table 3 indicates, this degree of aortic constriction affected specifically the systolic phase of the blood pressure causing an average decrease of 77.5 per cent in the pulse pressure. Despite the fall in the mean and pulse pressure, it will be observed that the average renal blood flow in the five dogs showed a slight increase (average increase, 6 per cent) following the reduction in the renal blood pressure by aortic constriction, although this increase was not considered significant. The inulin clearance and filtration fraction fell immediately after the constriction as had already been observed in the acute experiments. But when these five dogs were allowed to survive, it was found that four of them developed a chronic hypertension (see table 3), usually manifested as a rise in the diastolic pressure one to two days following the application of the clamp. The production of hypertension has been reported (20) to occur following constriction of the aorta above the renal arteries, and Steele (21, 22) pointed out that in this type of hypertension the diastolic increased more than the systolic phase, if the pressure were obtained from the femoral artery.

In three of these chronic dogs, the diodrast and inulin clearances were repeated and, although two of these dogs (3-c, 4-c) were hypertensive at the time of the determinations, there was no significant renal ischemia present in the three dogs examined. There was, however, an increase in the inulin clearance in two, and an increase in the filtration fraction in all three.

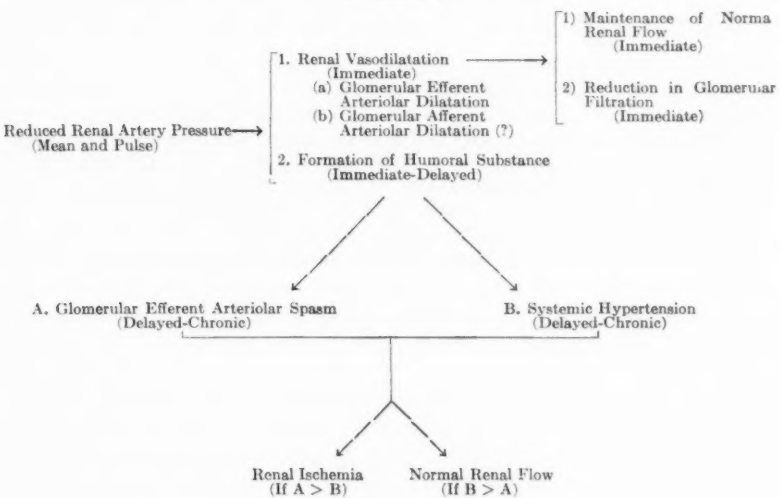
Discussion. The observations obtained from the experiments described above, indicate that a reduction in the renal artery pressure (both mean and pulse), is followed by a renal vasodilatation, and a fall in the glomerular filtration rate. If the fall in pressure is not too great, there will be no immediate ischemia. During this period of reduced renal artery pressure and glomerular filtration rate, a humoral substance, capable of neutralization by a kidney with normal hemodynamics, appears to be formed. This substance in turn apparently effects a glomerular efferent arteriolar spasm causing a delayed ischemia which occurs even after the release of the aortic constriction. It is important to point out that renal ischemia does not appear necessary for the production of this substance.

Hypertension was produced in four out of five dogs by moderate constriction of the aorta above the renal artery orifice. However, no ischemia was observed either immediately after the constriction of the aorta or three days later when a systemic hypertension was present. *From these observations it appears that renal ischemia is neither the initiating nor the maintaining factor in experimental hypertension.*

In all the dogs, however, there was evidence of increased glomerular efferent arteriolar spasm, and it was believed that the renal ischemia occur-

ring secondarily in the acute experiments was absent in the chronic experiments because of the rise in the systemic blood pressure in the hypertensive dogs. In other words, it is highly probable that renal ischemia in experimental hypertension of this type is not only a secondary phenomenon, but may even be absent if the systemic pressure rises high enough to overcome the increased glomerular efferent arteriolar spasm produced by the humoral substance of the deranged kidney. Considered from this viewpoint, the presence or absence of renal ischemia in experimental hypertension is dependent upon the ratio of the amount of glomerular efferent arteriolar spasm to the intensity of the systemic hypertension. The following diagram illustrates the probable changes occurring in experimental hypertension as suggested by our results.

DIAGRAM 1



SUMMARY AND CONCLUSIONS

1. The renal hemodynamics and systemic blood pressure were studied following aortic constriction above and between the renal artery aortic orifices.

2. It was found that renal ischemia is not necessary for the initiation or maintenance of a chronic (renal) experimental hypertension.

Note: The authors wish to express their thanks to P. Blakesley, H. L. Newman, E. MacNaughton and J. Smith for their aid in the execution of this problem.

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THE EFFECT OF DESOXYCORTICOSTERONE ACETATE AND OF BLOOD SERUM TRANSFUSIONS UPON THE CIRCULATION OF THE ADRENALECTOMIZED DOG[1]

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I. *The Effect of Desoxycorticosterone Acetate upon Blood Pressure and Plasma Volume.* Potent adrenal cortical extracts, even in large amounts, will not induce either a transient or persistent elevation in blood pressure above normal levels in man or animals (1). Within recent years, however, several clinical investigators have reported that the synthetic adrenal hormone D.C.A. (desoxycorticosterone acetate) may cause hypertension in the Addison's disease patient (2, 3, 4, 5). Elevations in pressure have also been observed in normal dogs and rats following its use (1, 6). Since the Addison's disease patient on D.C.A. therapy also shows a striking increase in plasma volume (2, 3, 4), the elevation of pressure might be assumed to be dependent in some manner upon the volume rise. The first section of the present study is concerned with this possible relation between blood volume and pressure changes in adrenalectomized and intact dogs given D.C.A.

Blood pressures were determined by the intra-arterial needle puncture method (7) without anesthesia, all animals being table trained. Plasma volumes were measured by the blue dye T-1824 (8). The dogs were fed a constant diet of Ken-L-Ration with a supplement of 2 grams NaCl. With this dietary regime, the maintenance dose of D.C.A.[2] was from 0.25 to 0.5 mgm. per dog per day, in the interval between experiments.

A. *Blood pressure.* Daily injections of D.C.A. induced, after a lag period of about 48 hours, a slow but progressive rise in arterial pressure, reaching a peak within 6 to 13 days (table 1, fig. 1). With continued treatment, the pressure might show a slight regression or be maintained at this peak level. In all cases it stabilized at some 20 to 30 mm. Hg above the normal.

[1] Part of the expenses of this investigation were defrayed by Julian M. Livingston of New Rochelle, N. Y.

[2] The desoxycorticosterone (percorten) used in these experiments was supplied through the generosity of Ciba Pharmaceutical Products, Inc.

When cortical extract was substituted, the pressure slowly declined to normal or near normal within 7 to 10 days.

Of seven adrenalectomized dogs studied, we have observed but one which failed to show this elevation in blood pressure (dog 7, table 1). One other animal, observed through four continuous cycles of D.C.A. therapy and cortical extract substitution, failed to show a significant rise in pressure on the third cycle, but did show a full rise later (fig. 3). With the possible

TABLE 1

Blood pressure and plasma volume changes in adrenalectomized and intact dogs placed on D.C.A. therapy

DOG	ON CORTICAL EXTRACT			ON D.C.A.					
	Plasma volume	Plasma volume	Blood pressure	Dosage	Plasma volume	Plasma volume	Time to vol. peak	Blood pressure	Time to pressure peak
Adrenalectomized dogs									
	cc.	*cc. per kgm.*	*mm.* Hg	*mgm.*	*cc.*	*cc. per kgm.*	*days*	*mm.* Hg	*days*
1	490	46.2	102	0.5	591	54.7	4	126	10
2	420	46.2	100	0.5	588	60.6	4	120	6
3	721	55.9	104	1	848	61.4	4	129	13
4	500	50.0	100	1	799	74.0	6	147	10
5	459	52.5	100	2	630	70.8	5	122	8
6	568	44.4	104	5	732	55.0	6	118	8
7	597	55.3	105	5	656	60.2	7	110	13
Ave.	536	50.1	102		692	62.4	5	124	10
Intact dogs									
1	659	46.4	109	2	708	52.8	12	128	12
2	614	57.4	111	2	701	60.9	10	120	10
3	485	54.8	112	4	552	67.3	14	120	14
4	621	56.5	108	5	632	60.0	8	108	
Ave.	595	53.8	110		648	60.2		119	

exception of this latter animal, a positive correlation between D.C.A. dosage and the extent of the pressure rise was not observed (table 1).

The dog with intact adrenal glands, given equivalent daily doses of D.C.A., also shows a definite tendency toward an elevated blood pressure, but the change was neither as marked nor as regular as in the adrenalectomized animals (table 1 and fig. 2).

B. *Plasma volume.* The adrenalectomized dog placed on D.C.A. therapy showed a plasma volume gain within the first 24 to 28 hours. A definite peak was reached in 3 to 7 days, after which, despite the continuance of D.C.A. therapy, the volume usually declined, to stabilize at 5 to 10 cc.

per kgm. body weight above the normal (fig. 1). An occasional dog has shown a spontaneous decline to normal after 20 to 30 days of D.C.A. therapy. The plasma volume changes have not appeared to be necessarily correlated with the dosage employed.

The intact dogs were apparently not as sensitive to D.C.A. as the dog lacking adrenals in so far as plasma volume increases are concerned (fig. 2). Two of four dogs studied showed plasma volume increases, while the others revealed no change despite prolonged treatment with relatively

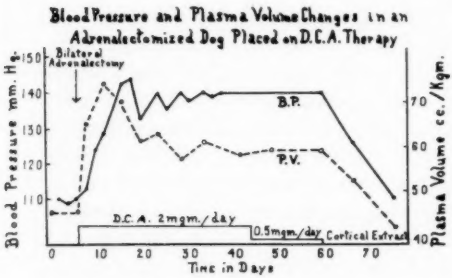

Fig. 1

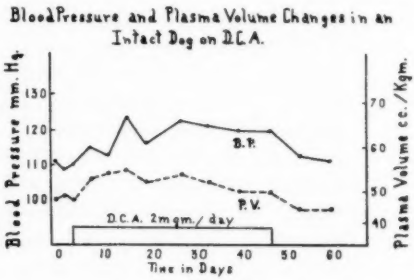

Fig. 2

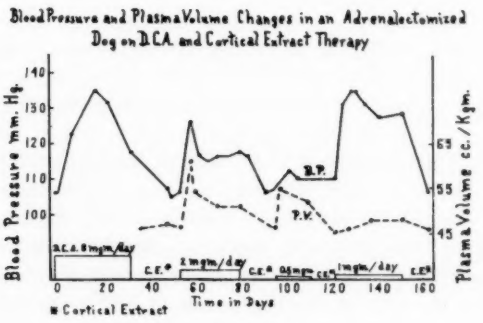

Fig. 3

large doses. One of the two dogs which failed to respond, was later adrenalectomized, and treated continually with a D.C.A. dosage of 5 mgm. per day. In spite of this high dosage, still no change in volume was observed (dog 4, table 1).

It might be assumed that the persistent elevation in blood pressure which follows use of D.C.A. therapy was dependent upon the rise in plasma volume. That this is not necessarily true is indicated by several facts: 1, the plasma volume rise always precedes the pressure rise; 2, by the time the peak of the pressure rise has been attained, the plasma volume has

significantly declined; 3, the plasma volume may increase without an accompanying pressure rise, and we have also observed dogs which exhibited a well marked pressure rise without significant change in the plasma volume (fig. 3, cycles 3–4); 4, in those cases where D.C.A. therapy is long continued, the plasma volume may decline to normal while the pressure remains high; 5, when cortical extract is substituted for D.C.A., the plasma volume has invariably returned to normal, while the pressure may remain somewhat above normal. It would seem, therefore, that the factors responsible for the persistent high blood pressure are more complex than a simple dependence of the blood pressure upon the plasma volume.

Grollman, Harrison and Williams (1) have suggested that the hypertensive effect of D.C.A. may be due to a toxic action of steroids on the kidney. This idea is not supported by these experiments, however. 1. The adrenalectomized dog receiving D.C.A. usually shows a return to normal blood pressure levels when cortical extract is substituted. Study of electrolyte concentrations, blood volume, and hemoconcentration, have revealed that the pressure fall was not due to inadequate extract dosage. Return to normal pressure levels was observed in all except one dog, and the length of time the animal had been receiving D.C.A. and the number of times the pressure had been elevated, seemed not to affect the outcome (fig. 3). 2. The intact dog exhibits a smaller response to D.C.A. than does the adrenalectomized animal. 3. In the face of a possible renal damage, one might perhaps expect a rise in blood urea nitrogen with D.C.A. therapy, whereas the reverse is usually true. It would seem that kidney damage is not of primary importance in producing the persistent elevation of pressure.

II. *The Effect of Transfusing Blood Serum from Normal Dogs into Adrenalectomized Animals.* The writers have repeatedly called attention to certain disabilities of the peripheral circulation of adrenalectomized dogs not receiving cortical hormone therapy. Among other changes, these animals appear to show marked atony of the capillaries resulting in peripheral stagnation, pooling of blood, anoxemia and increased permeability (9, 10, 11, 12). Much of the evidence for this view has been of an indirect nature. In the following experiments, increased permeability of the capillaries of the adrenalectomized dog apparently can be readily demonstrated. The experiments reveal, in a striking manner, the effect of cortical extract and D.C.A. in restoring to normal the capillary circulation.

Sterile blood serum, collected from large normal dogs, was infused into the jugular vein at a rate of 3 to 4 cc. per minute, the temperature of the serum being maintained at 37°C. throughout. Wherever possible serum obtained from the donor animal was divided into two portions and given to an adrenalectomized and a normal control recipient. The interval between withdrawal of blood and transfusion of the serum was approximately fifty minutes. The amount of serum given was 160 cc.

A. *Adrenalectomized dogs receiving daily maintenance doses of cortical extract.* Both intact dogs, not receiving therapy of any kind and the active, vigorous, adrenalectomized animal, receiving daily maintenance doses of cortical extract show no symptoms of circulatory failure or signs of edema when given serum transfusions. A representative case of the series of adrenalectomized dogs receiving maintenance extract is shown in table 2 (dog 4). The blood pressure rose while the transfusion was in progress but declined to the normal level within two hours. Hematocrit and hemoglobin values indicated a hemodilution at the end of the transfusion, of the order to be expected if the injected serum was largely retained in the circulation. At the end of two hours both hemoglobin and hematocrit had returned to pre-transfusion levels. The evidence seems perfectly clear that both the intact dog and adrenalectomized animal receiving adequate maintenance extract do not differ in their responses to serum transfusion.

B. *Dogs showing mild adrenal insufficiency.* Six dogs were used in this study. The daily maintenance injections of cortical extract, or D.C.A., were discontinued and the animals permitted to develop mild degrees of insufficiency. The interval between the time of extract withdrawal and development of symptoms varied, but was considerably longer for those animals maintained on D.C.A. in oil for an extensive period of time than for animals receiving extract.

With the exception of dog 1, table 2, all animals were eating full rations when used as recipients for the transfused serum. When the arterial pressure had declined to 75 to 80 mm. Hg the transfusions were started. At that time the average plasma volume loss, as determined by the dye method, was 125 cc. per dog. The average loss as derived from hematocrit and hemoglobin values was 190 cc. Therefore, a transfusion of 160 cc. was used in all cases.

The animals (table 2) showed a pressure rise varying from 5 to 15 mm. Hg during the first stage of the transfusion, which usually persisted until 80 to 100 cc. of serum had been injected. In two cases, the pressure remained at the initial level or above until the end of the transfusion (table 2, dog 2). In four cases, it had declined below the starting level by the conclusion of the transfusion. Regardless of whether the arterial pressure was at the starting level or not, it invariably declined to shock levels within 2 to 7 hours. Five of six animals exhibited circulatory failure within 2 hours of the completion of the transfusion, so that it was necessary to administer cortical extract to revive them.

The transfused adrenalectomized animals all developed edema, especially marked around the eyes, lips and ears. Two animals also showed edema of the legs, abdominal wall and scrotal sacs. One dog which had previously received an injection of dye for a plasma volume determination, exhibited blue stained edematous areas scattered over the body. The

TABLE 2

The effect of transfusing normal blood serum into adrenalectomized dogs

DOG	DATE	TIME	BLOOD PRESSURE	PULSE	HEMATOCRIT	HEMOGLOBIN	REMARKS
			mm. Hg	*per minute*	*per cent*	*gm. per cent*	
Dog 1 13.4 kgm.	2/14	10:00 a.m.	114	68	32.2	13.6	Discontinued maintenance D.C.A. therapy
	2/23	3:50 p.m.	68	52	53.0	18.5	Started serum transfusion
		4:30 p.m.	56	112	56.1	19.2	Finished transfusion (160 cc.). In collapse, massive edema eyes and lips
		6:45 p.m.	52	68			Edema more severe
		11:30 p.m.	47	72			Complete collapse. Given 5 mgm. D.C.A.
	2/24	9:30 a.m.	57	72			Stronger, edema disappeared. Given 2 mgm. D.C.A. per day
	2/27	9:30 a.m.	102	92	37.0	14.7	Normal
Dog 2 13.1 kgm.	5/31	10:00 a.m.	102	80	29.0	9.1	Discontinued maintenance cortical extract
	6/5	6:00 p.m.	75	112	45.2	10.8	Started serum transfusion
		6:42 p.m.	90	96	41.8	10.2	Finished transfusion (160 cc.). One hour later dog showed progressive edema of face, neck and legs
		8:45 p.m.	46	88	46.2	11.3	Complete collapse. Marked edema. Injected cortical extract
	6/6	12:15 p.m.	75	104	34.2	9.9	All symptoms and edema disappeared. Started second serum transfusion
		12:45 p.m.	102	84	30.9	8.9	Finished transfusion (160 cc.). No symptoms
		3:00 p.m.	108	78	32.8	9.0	Normal, no symptoms or edema
Dog 3 10.7 kgm.	6/7	10:00 a.m.	104	68	44.1	13.5	Discontinued maintenance cortical extract
	6/10	3:20 p.m.	74	124	47.1	13.8	Started serum transfusion
		3:45 p.m.	87	146			Received 80 cc.
		4:00 p.m.	58	116	49.8	14.2	Finished transfusion (160 cc.). Marked edema of face, legs and scrotum
		6:00 p.m.	46	132	52.4	15.8	Very weak, marked edema. Injected cortical extract
		9:40 p.m.	82	104	48.2	14.1	Marked improvement, edema almost disappeared

TABLE 2—*Concluded*

DOG	DATE	TIME	BLOOD PRESSURE	PULSE	HEMATOCRIT	HEMOGLOBIN	REMARKS
			mm. Hg	*per minute*	*per cent*	*gm. per cent*	
Dog. 3—*Cont.*	6/11	1:45 p.m.	84	76	40.6	12.6	Edema almost gone, started second transfusion
		2:30 p.m.	88	76	50.3	13.5	Finished transfusion (160 cc.). Depressed, no further edema
		4:30 p.m.	84	100	49.0	13.5	No symptoms, active
	6/12	9:35 a.m.	98	80			Normal
Dog 4 9.5 kgm.	6/6	3:10 p.m.	112	70	30.2	8.3	On maintenance cortical extract. Started transfusion
		3:50 p.m.	122	80	23.8	6.6	Finished transfusion (160 cc.). No symptoms
		5:40 p.m.	110	76	29.9	8.1	No symptoms, no edema, normal

edema showed significant decrease within 2 hours after cortical extract was administered intravenously and, with the exception of one animal, had completely disappeared within 24 hours.

Only two dogs showed a hemodilution, slight in extent, at the end of the transfusion. The other four dogs either failed to show dilution or actually showed a blood concentration, and all dogs showed hemoconcentration two hours after the completion of the transfusion. There was no evidence that the transfused serum remained in circulation; on the contrary, the evidence all pointed to the conclusion that even much of the serum in circulation previous to the transfusion had also been lost.

Two of the transfused dogs died; the others were restored to normal health by cortical extract or D.C.A. injections when they were in collapse. The animals receiving extract made a very rapid recovery with demon strable improvement in the circulation within 2 hours. Since D.C.A. in oil, injected intramuscularly, required a longer time for absorption, the recovery of D.C.A. treated animals was less dramatic (table 2, dog 1).

C. *Transfusion of extract treated adrenalectomized dogs during recovery from circulatory collapse.* Three dogs which had received serum transfusions on the previous day, and had developed circulatory collapse necessitating administration of cortical extract, were again transfused during the recovery phase. At this time the blood pressure had risen from shock levels to about the same level to which it had fallen prior to the first transfusion (table 2, dogs 2 and 3). After the second transfusion, no signs of

circulatory embarrassment or edema appeared. The blood pressure of two animals rose to, and remained normal throughout the transfusion and thereafter, and the blood was diluted as evidenced by decreases in hemoglobin and hematocrit levels (table 2, dog 2). One animal (table 2, dog 3) of this series showed some edema still persisting from the transfusion of the previous day, when given the second serum transfusion. The animal responded to the transfusion without aggravation of the edema or evidence of strain on the circulation, although some hemoconcentration took place.

The chief point of interest is that when cortical hormone was present, even though the blood pressure was low at the beginning of the transfusion, the response of the animal was essentially similar to that of the intact dog and quite different from the response seen the day before in the same animal, when no hormone was available.

Discussion. The fact that potent cortical extract will not cause elevation of blood pressure above normal, indicates that the efficiency of D.C.A. in this respect is due to some intrinsic property of this steroid. Other steroids of the cortex are not known to produce this effect, in the dog, at any rate. However, Grollman, Harrison and Williams (1) state that the "hypertensive" effect of D.C.A. is not specific for the normal rat. Although Reichstein and Euw (13) isolated D.C.A. from cortical extracts, it is apparently present in smaller quantities than the other steroids known to possess physiological activity. The extremely low concentration of D.C.A. in crude extract may perhaps explain the inability of the latter to cause persistent elevation of the pressure above normal. The writers have elsewhere (9, 10, 11, 12) presented evidence that D.C.A. is highly effective in maintaining the functional integrity of the peripheral vasculature of the adrenalectomized dog. It apparently prevents atony of the arterioles and capillaries and seems to maintain the normal permeability of the latter. D.C.A. when used as a prophylactic fore-treatment, will prevent the circulatory failure which invariably follows various shock inducing procedures in adrenalectomized dogs, and will restore to normal the collapsed circulation once it has developed (12, 14). It seems possible, therefore, that this steroid may induce persistent "hypertension" in adrenalectomized dogs by reason of the cumulative action of several factors: 1, increase in plasma volume; 2, increase in the inherent tone of the peripheral vessels.

Evidence from the serum transfusion experiments indicates that cortical hormones are probably concerned with maintenance of normal capillary permeability. It is surprising that transfused serum should so rapidly leak through the capillaries of the adrenalectomized dog carrying with it much of the animal's own blood fluid, thereby leading to rapid circulatory failure and edema. Since such changes do not occur in the transfused intact dog, or the adrenalectomized animal receiving maintenance extract, and are readily corrected by cortical hormones, it is apparent that they

must be due to cortical hormone deficiency somehow affecting the permeability of the capillaries. However, there is always the possibility that the adrenalectomized dog not receiving extract, and without hormone reserves, is peculiarly sensitive to normal blood serum or perhaps to hypothetical substances which might form during the course of its preparation for transfusion. Menkin (15) has shown that cortical extracts, wholly or in part, will inhibit the effect of substances such as leukotaxine which increase capillary permeability in intact animals.

SUMMARY

1. Potent adrenal cortical extracts will not induce elevation of blood pressure above normal levels in either intact or adrenalectomized dogs.

2. Desoxycorticosterone acetate causes persistent elevation above normal of both blood pressure and plasma volume of the adrenalectomized dog. The effect of comparable dosage upon blood pressure and plasma volume of the intact dog is less consistent and striking.

3. There appears to be little if any correlation between D.C.A. dosage employed and extent of the blood pressure rise when doses of more than 0.5 mgm. per dog per day are used.

4. The blood pressure rise which follows D.C.A. therapy in adrenalectomized dogs, is usually associated with an increase in plasma volume, but the blood pressure elevation is not necessarily dependent upon the increased plasma volume.

5. Transfusion of blood serum of normal dogs into other normal dogs and into adrenalectomized animals receiving adequate maintenance doses of cortical extract or D.C.A. does not induce symptoms of any kind. Similar transfusions of serum into adrenalectomized dogs not receiving extract or D.C.A. therapy promptly lead to circulatory collapse accompanied by edema. Injection of extract causes disappearance of edema within a few hours and the blood pressure slowly rises to normal levels with disappearance of all symptoms.

6. Adrenalectomized dogs with lowered blood pressure can be transfused with serum without circulatory embarrassment or signs of edema if given cortical extract or D.C.A. before transfusion.

7. The experiments on serum transfusion offer supportive evidence for the view that the permeability of the capillaries is markedly increased in the adrenalectomized dog not receiving extract and that cortical hormones restore the permeability of these vessels to normal.

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THE SEQUENCE OF FRACTIONATE CONTRACTION AT DIFFERENT SURFACE REGIONS ON THE RIGHT AURICLE AND VENTRICLES OF THE DOG'S HEART[1]

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It has been recently shown that the occurrence of fractionate contraction at different regions on the surface of the dog's heart is coincident, within the limits of error of measurement, with the main peak of the differential potential-time curve recorded from the same region[2] (1). The differential curve thus offers an accurate means for mapping the sequence of onset of contraction at different surface regions. This report is concerned with the determination in this way of the sequence of onset of contractions on the surface of the right auricle and the ventricles of the dog's heart.

METHODS. The differential electrodes used were made up of two zinc zinc-sulphate electrodes provided with a common wick and mounted close together. The wick was held stretched in the form of a V and in contact with the heart surface by means of a thread passed through it. The electrode was connected through a direct current amplifier to a cathode ray oscillograph. A reference curve was recorded simultaneously by means of a unipolar lead, usually from the apex of the right auricle or the apex of the left ventricle, connecting to a second amplifier and oscillograph. Recording was made on 35 mm. unperforated film, driven at a speed of 110 mm. per second. Measurements were made in a comparator with 40× magnification.

RESULTS. The relative times of occurrence of the main differential peak from various surface regions on the anterior surface of the right auricle and the anterior surface of the two ventricles are given in the figure. In each case they represent the average from the data of seven experiments. Very definite differences in time of onset of fractionate contraction in different regions are apparent. The interval between the first and last regions involved is of the order of 0.03 sec. for the auricle and 0.02 sec. for the ventricles. The first regions entering into fractionate

[1] Supported in part by a grant from the Wisconsin Alumni Research Foundation.

[2] The term "fractionate contraction" was introduced by C. J. Wiggers (2) to designate local contraction of regions of heart muscle as distinguished from contraction of the chamber as a whole, as recorded by the usual suspension methods.

contraction on the surface of the right auricle are those contiguous to the upper part of the sulcus terminalis and from here contraction proceeds in rather regular fashion to the appendage and downward to the auriculo-ventricular junction. In the ventricles, the first surface contractions appear on the right ventricle contiguous to the inter-ventricular groove. Other regions enter into contraction in a manner which fails to show any evidence for a progressive involvement such as exists in the right auricle. In general, the surface of the right ventricle becomes involved before that of the left, with the exception of the conus of the pulmonary artery, which always enters into contraction late.

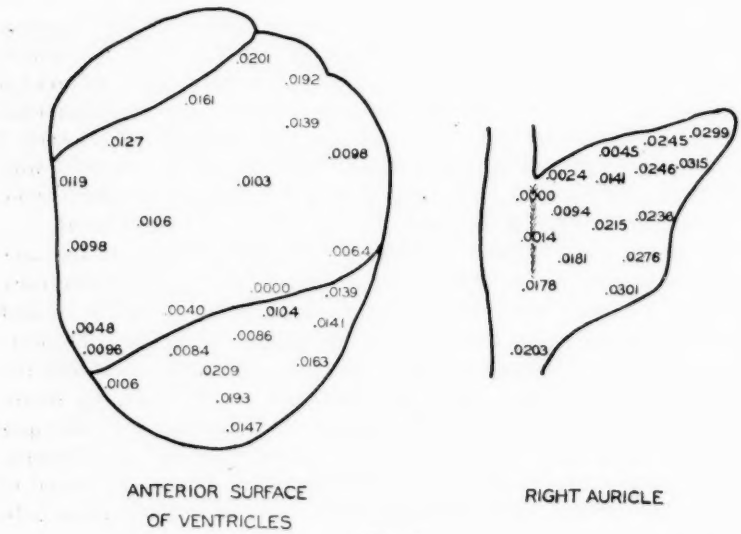

Fig. 1

We have also recorded, in a few experiments, the relative time of occurrence of the main differential peaks from the posterior surface of the ventricles. These follow the same general pattern as on the anterior surface-indicating early contraction along the inter-ventricular sulcus and involvement in general of the right before the left ventricle.

Discussion. Previous work in this field has been concerned with attempts to determine an electrical state which signals the occurrence of the "impulse" of "excitation process" which presumably precedes the onset of contraction. The criterion which has been employed in nearly all of this work has been the assumption that the occurrence of "excitation" is coincident with a local fall of potential or state of "negativity." This

electrical state was supposed to spread over the muscle in the form of a "wave of negativity" coincident at all regions with the "impulse." Several different methods have been employed by workers in this field. In many cases bipolar leads from two separate regions on the heart surface have been used, in spite of the fact that the fallacies inherent in this method are obvious (3). Bipolar curves are composites of the potential changes under each electrode and it is impossible to resolve them into their two components. A few workers have used unipolar leads (4, 5), the indifferent electrode being placed on the chest wall or on a leg. The relative times of occurrence of maximum negativity at various regions were determined by reference to a constant curve, usually an electrocardiogram, recorded simultaneously. In efforts to determine the maximum state of electrical negativity in a region, the "monophasic action current," obtained from leads from an injured and an uninjured region on the heart surface, has been used (6) under the false assumption that the curve results from the development of a negative electrical state at the uninjured region (7).

The differential electrode, of the type employed by us in the present work, was first suggested and employed by Clement (8) and later by Erfmann (9). The current flow in the differential electrode is small and it was difficult to obtain satisfactory curves with the unamplified string galvanometers, which these investigators employed. The criterion used for the occurrence of excitation was apparently the start of the differential curve, but deductions as to the spread of the "wave of excitation" were made from the form of the recorded curves.

It is obvious from recent work that we have no proven criterion at present for the determination of the onset of the state of excitation, if it is assumed that this state precedes the onset of contraction. The onset of fractionate contraction in a region is however accompanied by an electrical state defined by two types of electrical curves that may be recorded, the unipolar and differential potential-time curves. The onset of contraction is coincident or nearly coincident with the occurrence of the most rapid time rate of change of potential in the region as indicated by the unipolar curve and by the maximum flow of electrical current as indicated by the maximum potential of the differential curve. In practice the latter is more easily identified and is the preferable curve for the determination of the sequence of occurrence of fractionate contraction at different regions of the heart surface. It is to be noted that the *start* of these curves is theoretically simultaneous from all parts of the heart and is found to be so provided sufficient sensitivity of the recording apparatus is employed. The occurrence of the events on these curves noted above as associated with fractionate contraction in the region occurs at different times in different regions. Presumably, the state of "excitation" in a region begins at some instant which precedes the occurrence of the maximum time rate of

potential change, as indicated by the unipolar curve and the occurrence of the maximum peak of the differential curve, but we do not at present know what point, if any, on these or any other electrical curves recorded from the heart, signals this instant. There is certainly no valid reason to associate it with maximum "negativity" or "positivity" of the region, since either state may precede or follow the onset of fractionate contraction in the region.

SUMMARY

The sequence of occurrence of fractionate contraction at the different surface regions of the right auricle and ventricles of the dog's heart is determined by recording differential potential-time curves from the various regions along with a constant reference curve. The results are indicated in the accompanying figure.

The fact that no criterion is available at present which can be used to determine the instant of "excitation" of a region of heart muscle, is brought out and discussed.

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ON THE MECHANISM OF ENHANCED DIABETES WITH INFLAMMATION[1]

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The effect of an infection or inflammation on human diabetes is well known to clinicians and pathologists. In brief, the course of the disease is considerably accentuated, and to some extent the inflammatory reaction is intensified. There is also some evidence of a generalized fall in resistance as indicated by an augmented susceptibility to infection. The mechanisms involved to explain the enhancement in the diabetic condition as well as the increased severity of the local inflammation have never been satisfactorily elucidated.

There is evidence that the healing of surgical incisions is delayed when the treatment of diabetes is inadequate. This question has been recently studied by Bennett in depancreatized dogs subsequently deprived of insulin (1, 2). The rapid course assumed by pulmonary tuberculosis in diabetic patients is common knowledge (3). Sweet has pointed out that the serum obtained from dogs in the end stages of diabetes loses some of its normal bactericidal power (4). More recently Richardson has reported that the complement in the blood of diabetic patients does not seem to differ in amount from that of the blood in non-diabetics. This has been found true irrespective of whether or not infection is present. The antibacterial power of the blood of diabetic patients seems, however, reduced. Finally this investigator has found in diabetics inoculated with typhoid vaccine a diminished capacity for agglutinin formation (5). The nutritional state of experimental animals as exemplified by depleted liver glycogen seems to be concerned with the development of a lowered agglutinative titre after administration of typhoid vaccine (6). The organs of

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[2] In the course of this study technical aid was also received from Miss Irene Lapouse and from Miss Joan Malkenson.

depancreatized cats after cutaneous inoculation of bacteria show the presence of the microörganisms with greater frequency than the corresponding organs of normal controls (7). As a result of these various studies Richardson has concluded "that at least a part of the commonly recognized susceptibility of diabetics to infection might be concerned with this decreased power to form immune bodies as compared with normal individuals."

A recent survey of the literature by Perla and Marmorston (3) have led these authors to conclude that the hyperglycemia and the decreased sugar tolerance are apparently not the cause for the reduced antibody production. According to Wilder (2) resistance to infection is not diminished in a patient whose diabetes is well controlled and in whom nutrition is adequate. Nevertheless this author points out that a diabetic patient requires several times more insulin when infection intervenes. Rabinowitch infers that an insulin-destroying enzyme is involved, especially since pus cells are known to inactivate insulin *in vitro* (8). In this connection, Jensen, in a recent monograph, has reviewed the literature indicating that insulin can be inactivated by proteolytic enzymes (9). Greene and his collaborators (10) have recently made a comprehensive clinical study on the relation of delayed healing of clean and infected wounds to the height of the blood sugar level. They have concluded that there is no apparent connection between the height of the blood sugar level and the delayed healing of wounds or of infections in diabetes mellitus.

The present series of observations represents an attempt at elucidation of the basic mechanism concerned in explaining enhanced diabetes concomitant with inflammation. The results indicate that the excessive hyperglycemia seems referable to an increased proteolysis at the site of inflammation. The deamination of the split protein molecule favors the formation of a surplus of glucose which in turn gradually diffuses into the circulating blood stream. The enhanced local proteolysis induces severe tissue damage thus offering an explanation for the intensified degree of inflammation. The increased protein catabolism as well as the increased formation of sugar in the inflamed area can be readily controlled by administration of insulin.

EXPERIMENTAL. *The effect of inflammation on the blood sugar of depancreatized dogs.* An attempt was first made to reproduce as closely as possible the clinical diabetic condition with superimposed inflammation. All experiments were made on depancreatized dogs. It is, however, to be borne in mind in this connection that although there are many obvious points of similarity between the human form of the disease and this type of experimental diabetes, nevertheless there are also several notable differences. These have been recently reviewed by Long (11).

Blood samples were withdrawn from the heart of a series of dogs weighing from about 5 to 15 kgm. Blood sugar was determined by the Folin

method (12). Pancreatectomy was performed under nembutal anesthesia. All forms of nourishment with the exception of fluids in the form of skim milk or water were withheld from 12 to 24 hours following operation. After that time usually 50 grams of boiled lean horse meat mixed with 2 grams of sucrose and 2 grams of choline chloride formed for each of the two succeeding days the bulk of the solid food. Subsequently the ration of meat was gradually increased to about 150 or even as high as 300 grams per day. The sucrose was also increased to about 3 or 4 grams daily. In few instances raw pancreas was administered instead of choline chloride. Insulin administration began about 12 to 24 hours following pancreatec-

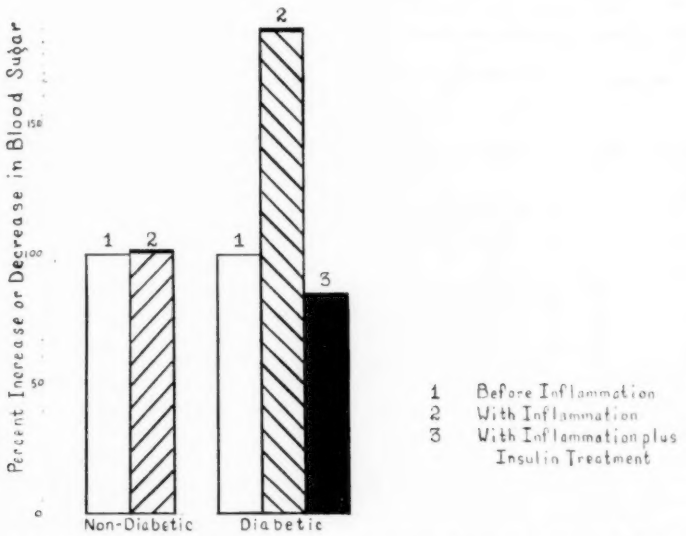

Fig. 1. Effect of inflammation on the blood sugar of non-diabetic and of diabetic dogs.

tomy. The dose of insulin consisted at first of about 3 units administered twice daily. The quantity of insulin was gradually increased to about 10 units daily. Blood sugar determinations were made almost daily. After a period, usually ranging from about 6 to 12 days, insulin administration was discontinued. After about 2 or 3 days the animal, under nembutal anesthesia, received an intrapleural injection of 1.5 cc. of turpentine. This, as described in previous studies, is a convenient method of inducing an acute inflammatory reaction accompanied by extensive exudation (13). At variable intervals, ranging from about 7 to 44 hours, blood samples were studied for their sugar content. Similar observations were made on

non-diabetic dogs in which a pleural inflammation likewise had previously been induced by the introduction of turpentine.

The results of all observations are summarized in table 1 and graphically illustrated in figure 1. It is clear that with a superimposed and extensive inflammatory reaction involving the whole of the right pleural cavity there occurs a sharp ascent in the blood sugar level. The average blood sugar in depancreatized animals is 253.0 mgm. per 100 cc. prior to the injection of the irritant. Following the injection of the irritant there is a rapid and sharp rise averaging 469.1 mgm. This is an average increase in blood sugar of 85.4 per cent. With administration of insulin throughout the

TABLE 1

The effect of inflammation on the blood sugar of diabetic and non-diabetic dogs

NUMBER		DURATION OF INFLAMMATION		DIABETIC DOGS		NON-DIABETIC DOGS	
Diabetic dogs	Non-diabetic dogs	Diabetic dog	Non-diabetic dog	Blood sugar prior to the introduction of the irritant	Blood sugar subsequent to the injection of the irritant	Blood sugar prior to the introduction of the irritant	Blood sugar subsequent to the injection of the irritant
		hrs.:mins.	*hrs.:mins.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*
1	9	7:20	6:23	311.7	497.3	120.3	95.9
2	9	9:20	24:50	224.1	269.2		118.1
3	10	15:45	10:40	257.1	522.2	112.9	83.8
4	11	24:30	23:00	240.9	412.4	71.4	74.8
5	12	25:15	23:10	163.3	312.8	69.8	55.6
6	13	25:18	24:45	257.4	630.9	81.7	111.1
7	14	33:32	24:50	335.3	442.9	63.7	64.2
8	15	14:00	25:05	234.0	482.0	69.3	70.8
8	16	44:15	42:45		652.5		108.1
	17*		6:15			81.0	57.2
Average				253.0	469.1	83.8	84.0

* Splenectomized two days prior to injection of irritant.

period of the experiment, as will be pointed out later on, this rise is wholly inhibited (fig. 1).

On the other hand, control non-diabetic animals with pleural inflammation fail to show any rise in blood sugar (table 1, fig. 1). Splenectomy was performed in one instance (see dog 17, table 1) to control for the operative procedure involved in pancreatectomy. The blood sugar level likewise failed to show any rise following the intrapleural injection of the irritant.

In figure 2 the rapid rise in blood sugar following the introduction of an inflammatory irritant in a depancreatized animal is compared with the course of the blood sugar level in a diabetic animal without any in-

duced pleurisy. It is clear that in the latter the blood sugar, though elevated, never reaches the hyperglycemic level encountered in a diabetic dog with a concomitant and severe inflammation.

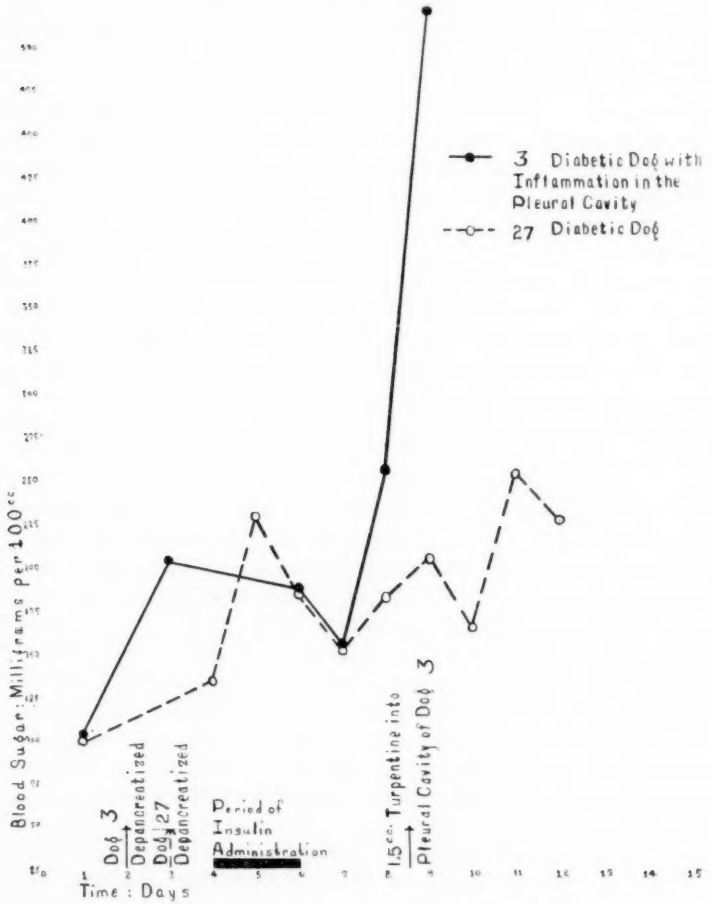

Fig. 2. Effect of inflammation on the blood sugar of a diabetic dog

These results seem to duplicate quite well the change in blood sugar level which one observes in a diabetic patient whose clinical course is complicated by infection.

Carbohydrate and protein metabolism in exudates of depancreatized dogs. What is the basic mechanism which induces a sharp rise in the blood sugar of diabetic dogs having a superimposed pleural inflammation? There

is one important feature of inflammation which must first of all be considered. One of the cardinal signs of this important manifestation of cellular injury, besides the other well-known criteria observed and described by the early writers, is the phenomenon of proteolysis (14).

The occurrence of protein digestion in inflammatory exudates has been known for a long time. The products of proteolytic digestion in exudates were recognized by Eichwald in 1864, who reported what he thought was a peptone in pus. Friedrich Müller (15) described the autolytic property of purulent exudates in tuberculosis and pneumonia. Opie (16) studied thoroughly the enzymatic property of cells of an exudate in digesting coagulated protein. According to this author the ability of phagocytic cells to remove injurious material is dependent on the possession of proteolytic enzymes. The importance of proteolysis in inflammation was again stressed by the writer in his recent studies on the isolation of leukotaxine (14, 17). By comparing both the amino acid nitrogen and the total proteins of exudates with the concentrations of these same constituents in the blood serum, he concluded that proteolysis forms a conspicuous feature of the inflammatory reaction.

The formation of glycogen from part of the protein molecule has been known for a long time. Claude Bernard believed in the possibility of such a conversion. The subsequent work of numerous investigators attested further the truth of this belief. The experiments of Wolffberg (18) and the studies of Voit (19) substantiated to a large extent this principle. The theory that in diabetes sugar originates in part from amino products was strongly advocated by Müller (20); but the definite proof was first afforded by Stiles and Lusk (21) and by Berger (22). Furthermore, the studies of Neuberg and Langstein indicated that in normal rabbits the ingestion of alanine was followed by the appearance of lactic acid in the urine (23). Ringer and Lusk (24) and Dakin and Dudley (25) found that in the phlorhizinized animal alanine is completely converted into glucose. In brief, these various studies demonstrated that cleavage of proteins through the process of deamination, is followed by the conversion of part of the protein molecule to glucose.

Lusk (21) and his collaborators, as well as Falta, Grote and Staehelin (26), found an enhanced protein metabolism in dogs rendered diabetic whether with phlorhizin or by pancreatectomy. Okada and Hayashi found in the blood of depancreatized dogs a hyperaminoacidemia (27). Similar studies on diabetic patients indicated likewise an increase in the amino nitrogen content of the circulating blood (28, 29, 30). The latter, however, could not be substantiated by Greene and his co-workers (31).

The studies cited above indicate that in an inflamed area there is active proteolysis. Furthermore in the diabetic animal with increased protein catabolism glucose can readily originate from amino products. Therefore

it seems reasonable to assume that in foci of proteolysis such as exist in inflamed areas, the process of protein breakdown in a diabetic animal might possibly be considerably enhanced. It seems quite immaterial whether one abides by the non-utilization or the over-production theory of diabetes (32). In either case gluconeogenesis from proteins could occur at the site of inflammation, thus allowing for the gradual diffusion of sugar into the circulating blood. The following series of experiments indicates that this indeed seems to be the state of affairs.

Blood samples were withdrawn from the heart of normal dogs. Chemical studies were undertaken to determine the level of lactic acid (33), sugar (34), total protein (35), non-protein nitrogen (36), urea (37), amino acid nitrogen (38) and pH (39). The analytical method utilized in each case is indicated in the bibliographical reference. Pancreatectomy was then performed under nembutal anesthesia. The post-operative care, the method and duration of insulin administration have already been described. Studies on the blood chemistry were repeated several days later but prior to the injection of the irritant in the pleural cavity.

Samples of both exudate and blood were withdrawn at varying intervals and chemical determinations of nitrogenous and carbohydrate constituents were made on both types of material. At the end of the experiment the animal was usually sacrificed and a necropsy performed. Several animals received about 8 to 10 units of insulin throughout the duration of the inflammation in an endeavor to determine the influence of this substance on proteolytic activity in an acutely inflamed area. Control studies were also performed on several non-diabetic dogs having an acute pleural inflammation. The results of all experiments are listed in tables 2 and 3. The data are graphically shown in figures 5 and 6. The results of a type experiment to illustrate the progressive change in protein metabolism in the exudate of a diabetic compared to that of a non-diabetic dog are presented in figures 3 and 4.

The data can be conveniently summarized as follows: The lactic acid of diabetic exudates averages 89.29 mgm. per 100 cc. as compared with 58.75 mgm. in non-diabetic exudates. This represents an elevation of 52 per cent. The administration of insulin in depancreatized animals completely inhibits the rise in exudate lactic acid, the average level being brought down to 57.84 mgm. per cent (table 3, fig. 5). The average concentration of sugar in diabetic exudates is 452.4 mgm. per 100 cc. of the material. The level in exudates of non-depancreatized dogs averages 78.87 mgm. There is thus an average increase of 473.6 per cent in the sugar content of diabetic exudates. The level is distinctly reduced with repeated insulin administration, the average figure being 191.7 mgm. of sugar per 100 cc.

The observations regarding the status of protein metabolism in exudates

TABLE 2

Studies on carbohydrate and nitrogenous metabolism in exudates of diabetic and non-diabetic dogs

DOG NUMBER		DURATION OF INFLAMMATION		CARBOHYDRATE METABOLISM				NITROGENOUS METABOLISM							
				Diabetic exudate		Non-diabetic exudate		Diabetic exudate				Non-diabetic exudate			
Diabetic	Non-diabetic	Diabetic dogs	Non-diabetic dogs	Lactic acid	Sugar	Lactic acid	Sugar	Total protein	NPN	Urea	Amino acid N	Total protein	NPN	Urea	Amino acid N
		hrs.: mins.	*hrs.: mins.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*gm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*gm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
1*	17†	7:05	6:15	89.1	422.9	119.3	44.1	3.7	74.1	72.0	9.7	4.4	38.0	29.0	8.9
3	11	15:45	23:00	167.3	422.8	40.6	73.8	4.1	84.7	127.0	12.1	3.5	40.2		6.9
18	12	16:35	23:30	47.0	419.8	30.2	86.2	3.6	69.1		12.0	4.8	49.0		5.5
4§	14	24:30	24:00	44.1	344.8	52.0	63.7	3.6	49.7		11.2	4.9	42.4		6.5
7	20	33:12	24:25	72.8	427.5	61.9	91.2	4.2	62.5	42.0	18.6	4.9	36.9		9.6
19	15	36:00	25:30	91.6	153.4	76.2	71.3	4.4	98.0		10.9	4.3	44.8		7.3
8	13	38:00	34:15	92.6	869.8	57.9	50.9	3.8	100.0		11.8	4.3	69.0		7.5
6	16	25:08	42:15	132.2	629.2	28.7	124.0	4.0	72.2	60.5	12.2	4.5	32.0	26.0	5.4
6	9	48:10	24:45	66.8	381.7‡	25.7	131.1	4.3	115.4	89.0	17.4	4.5	31.6	40.5	6.7
	9		47:50			95.0	52.4					5.3	41.7	42.5	9.6
Average				89.29	452.4	58.75	78.87	3.97	80.63	78.1	12.88	4.54	42.56	34.5	7.39

* Succumbed 25 min. after thoracentesis.

† Splenectomized several days preceding the injection of the irritant. Died 40 min. after thoracentesis.

‡ Fall in sugar may in part be referable to starvation.

§ Sample of exudate withdrawn from left pleural cavity, although irritant originally introduced into right thoracic cavity.

TABLE 3

Effect of inflammation on some of the carbohydrate and nitrogenous constituents of exudates in depancreatized dogs treated with low doses of insulin

DOG NUMBER	DURATION OF INFLAMMATION	LACTIC ACID	SUGAR	TOTAL PROTEIN	NPN	UREA	AMINO ACID NITROGEN
	hrs.: mins.	*mgm./100 cc.*	*mgm./100 cc.*	*gm./100 cc.*	*mgm./100 cc.*	*gm./100 cc.*	*mgm/100 cc.*
21	6:05	68.3	40.7*	5.9	21.3	14.0	3.9
22	6:20	43.6	55.7*	4.6	13.8	16.0	3.9
	27:50	21.8	251.0	4.0	47.2	34.5	12.7
23	7:23	71.3	303.1	4.0	22.0		6.0
	25:00	43.9	219.8	3.9	27.6		9.5
	47:10	31.7	383.1	3.6	40.2		10.6
19	11:30	124.3	88.5	5.4	54.0		9.4
Average		57.84	191.7	4.49	32.3	21.5	8.0

* Insulin administered only a few hours prior to removal of sample of exudate.

are of even greater interest. The average total proteins per 100 cc. of exudate in diabetic dogs is 12.56 per cent lower than that encountered in normal animals. The actual figures are 3.97 and 4.54 grams respectively

(table 2). In the exudates of depancreatized and insulin-treated dogs the average total protein concentration of 4.49 approximates that found in the non-diabetic (table 3). The products of protein catabolism show much more striking differences in the three groups of animals. The non-protein nitrogen of exudates in diabetic dogs averages 80.63 mgm. per 100 cc. of material. This is in sharp contrast to the average concentration of 42.56 mgm.

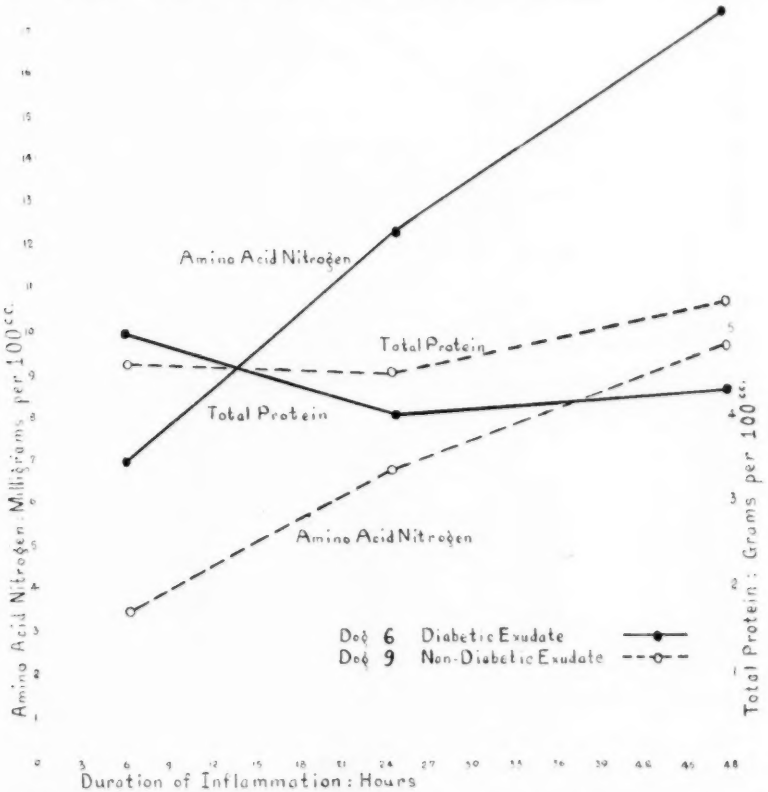

Fig. 3. Enhanced proteolysis in inflamed area of diabetic dog. Effect of experimental diabetes on amino acid nitrogen and total protein of exudates.

in the control non-diabetic preparations. A comparison of these figures indicates an increase of 89.45 per cent in the NPN of exudates of experimental animals. The urea in exudates of diabetic dogs averages 78.1 mgm. per 100 cc., whereas the exudates of control dogs yield an amount averaging 34.5 mgm. per cent. This represents a difference of 126.3 per cent. Finally the amino acid nitrogen concentration of diabetic exudates averages 12.88 mgm. per cent. The average amino acid nitrogen in con-

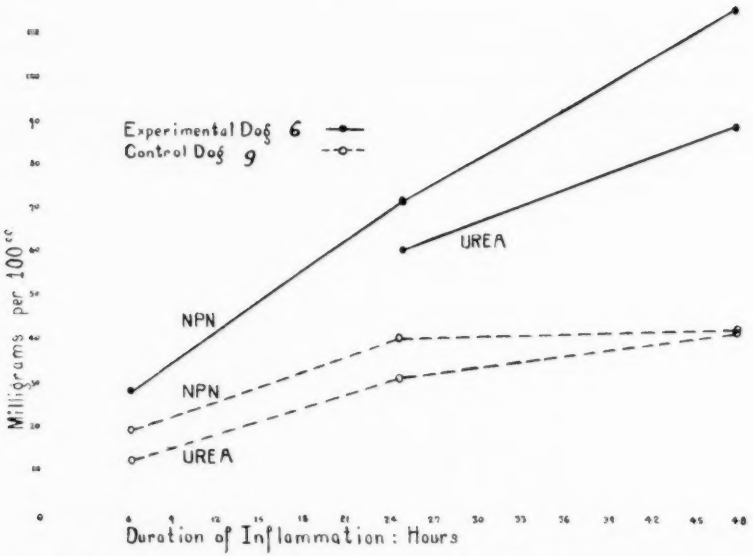

Fig. 4. Effect of experimental diabetes on non-protein nitrogen and urea concentrations of exudates.

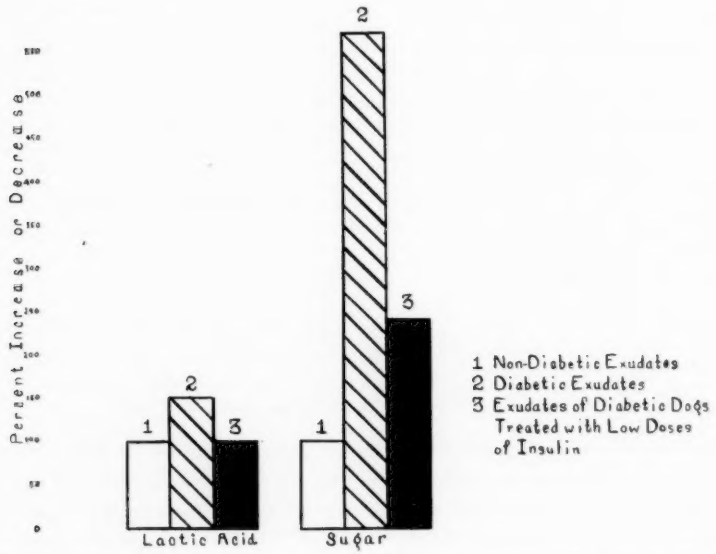

Fig. 5. Effect of inflammation on the carbohydrate constituents of exudates in diabetic and non-diabetic dogs.

trol exudates is found to be only 7.39 mgm. The average increase in the amino acid nitrogen of exudates in diabetic dogs is therefore 74.29 per cent. In brief, these figures on non-protein nitrogen, urea and amino acid nitrogen of exudates reveal a markedly enhanced degree of proteolysis at the site of inflammation of depancreatized dogs (table 2).

It is well known that insulin inhibits the formation of glucose from non-carbohydrate precursors (40, 9).

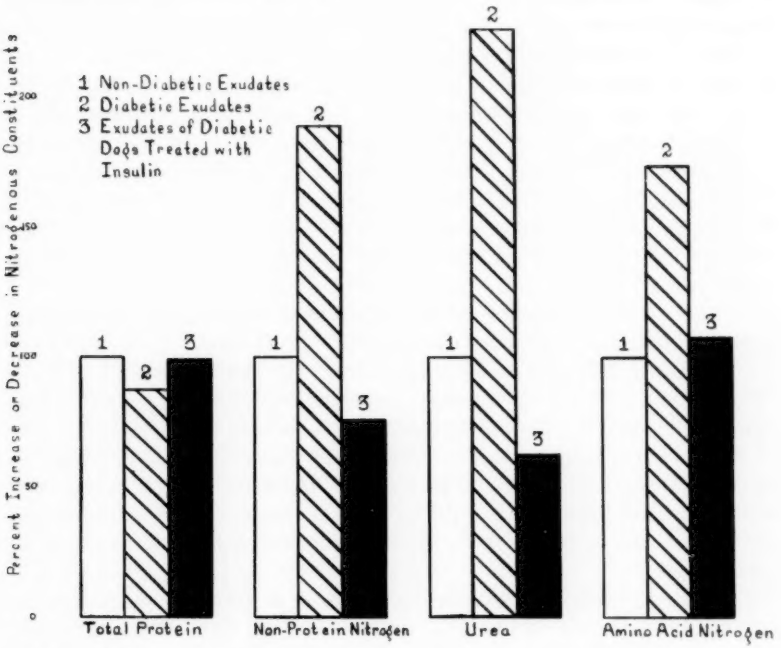

Fig. 6. Effect of inflammation on the nitrogenous constituents of exudates in diabetic and non-diabetic dogs.

There is evidence that the administration of insulin tends to lower the amino acid nitrogen of the blood. Luck and his associates showed that subconvulsive doses of insulin lower the amino acid content of the blood of dogs, rabbits, rats and man (41, 42). They were led to the conclusion that under the conditions of their experiments insulin seems both to increase the rate of amino acid catabolism and at the same time to inhibit the process of protein hydrolysis by which amino acids are generated. These observations on dogs were confirmed by Kerr and Krikorian (43); but Bischoff and Long were unable to substantiate the findings on rabbits (44). The studies were again followed up at a later date by Powers and

Reis (45). They confirmed the above-mentioned observations on rabbits reported by Luck and his collaborators; but they were unable to corroborate the additional claim that insulin tends to induce a rise in urea nitrogen. Farr and Alpert arrived at essentially the same conclusion (46). Subcutaneous injections of insulin produced sharp decreases in plasma amino acids with no significant changes in blood urea.

Bach and Holmes (47) reported that insulin decreased *in vitro* glucose formation by excised liver tissue. This reduction was accompanied by a diminution in urea formation. Stadie and his associates confirmed the inhibition of oxidative deamination by insulin on d-amino acids (48). Mirsky studied the influence of insulin on the protein metabolism of dogs (49). His data suggested that insulin exerts a nitrogen-sparing action by decreasing the rate of oxidative deamination in the liver, while at the same time it enhances the rate of amino acid utilization by muscles for protein synthesis. This investigator pointed out that "In experimental or clinical diabetes mellitus, insulin produces a profound decrease in urinary nitrogen excretion which is concomitant with an increase in carbohydrate retention."

The present series of observations indicates that pancreatectomized dogs with a superimposed pleurisy manifest not only a sharp rise in blood and exudate sugar but also a pronounced degree of proteolysis at the site of inflammation. If the excess sugar formation in the inflamed area is primarily derived from the local breakdown of proteins, the administration of insulin should both depress the level of glucose and inhibit the enhanced protein catabolism. Studies have therefore been undertaken in depancreatized dogs receiving twice daily about 8 to 10 units of insulin throughout the duration of a pleural inflammation induced as described previously.

The results of the experiments are shown in table 3. A convenient graphical comparison of the observations with the data obtained in non-diabetic and in diabetic dogs receiving no insulin during the period of the experiment is also shown in both figures 5 and 6. The results may be briefly summarized as follows: The average lactic acid of exudates in insulin-treated depancreatized dogs is essentially the same as found in non-diabetic dogs; the actual figures are 57.84 mgm. and 58.75 mgm. respectively. This indicates a significant decline in the lactic acid content when compared with the level in exudates of untreated diabetic animals. The average sugar of exudates likewise shows a marked drop in concentration; the content in the exudate of insulin-treated dogs being 191.7 mgm., as against 452.4 mgm. in untreated animals. When compared with the glucose level of exudate in control animals, the results represent an increase in sugar of 143.1 per cent in the insulin-treated group, and an increase of 473.6 per cent in the untreated diabetic dogs. It is to be noted

that in individual instances when insulin was administered a relatively short interval prior to removal of the exudate sample, the level of sugar was invariably very low (dogs 21 and 22, table 3).

The repression by insulin of glucose and lactic acid formation is likewise reflected in the protein catabolism. As already mentioned the average total protein concentration of exudate in the insulin-treated group is higher than that found in the untreated diabetic animals. It is essentially at the same level as encountered in control animals. The non-protein nitrogen and the urea of exudates are both considerably reduced by repeated insulin administration (table 3, fig. 6). The average contents are 32.3 mgm. and 21.5 mgm. respectively. This represents a decrease of 24.1 and 37.7 per cent when compared with the levels of these same constituents in exudates of control animals (table 3, fig. 6). This is in contrast to the marked elevation of the nitrogenous products in exudates of depancreatized dogs receiving no insulin (table 2). Finally, the average amino acid nitrogen of exudates after insulin administration is only increased 8.3 per cent in contrast to the significant rise of 74.3 per cent in exudates of untreated diabetic dogs.

In brief, the data show without any doubt that the enhanced proteolysis at the site of inflammation of diabetic dogs is completely held in abeyance by the administration of insulin. Since this fact is also paralleled by a corresponding appreciable reduction in glucose concentration, it is reasonable to infer, in view of what is known concerning the formation of sugar from proteins in diabetic animals, that the most likely source of gluconeogenesis at the site of inflammation is primarily protein in nature.

There is a further, though perhaps indirect, type of evidence in support of the view that the excess sugar and lactic acid formation in exudates is, at least in large part, probably derived from proteins. In earlier studies the writer has shown that the local acidosis which develops in an inflamed area is referable to glycolytic processes. The conversion of sugar to lactic acid was found to be the primary factor responsible for the gradual increase in hydrogen ion concentration at the site of an acute inflammation (13, 14, 52). The observations revealed a reciprocal type of relationship between the concentration in exudate of sugar and lactic acid. But in the present series of experiments on diabetic exudates, no such consistent relationship is detectable. The characteristic picture encountered is illustrated in figure 7. The reciprocal relationship between sugar and lactic acid, clearly manifest in the exudate of a non-diabetic dog (no. 9), is absent in the material withdrawn from the pleural cavity of a depancreatized dog (no. 6). This seems to indicate that in all probability the level of lactic acid in the exudate of a diabetic animal is not wholly conditioned by the sugar concentration. The graph clearly shows a parallelism between the level of these two substances rather than the usual type of

reciprocal relationship found in the exudate of a non-diabetic dog. It is therefore quite likely that this state of affairs is referable to an appreciable amount of sugar and lactic acid arising directly from protein breakdown.[3]

Carbohydrate and protein metabolism in the circulating blood of depancreatized dogs with a superimposed acute inflammation. The foregoing series of observations indicates that in a depancreatized dog a focus of inflammation manifests not only a disturbance in carbohydrate metabolism but also a concomitant and significant rise in protein catabolism.

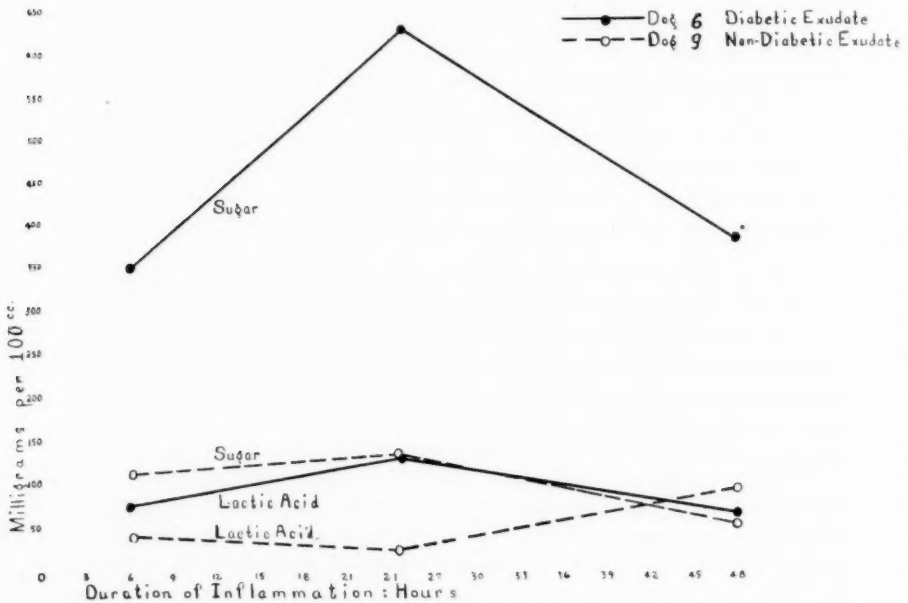

Fig. 7. Absence of reciprocal relationship between sugar and lactic acid in the exudate of a diabetic dog.

How do these changes affect the levels of the nitrogenous and carbohydrate constituents of the blood?

In the first place, is the high exudate sugar referable to a diffusion of this substance from the circulation? Two sets of evidences would tend to rule out such a conclusion. As pointed out in an earlier section, the

[3] Does any of the formed sugar in the inflamed area arise from fatty acids? This remains a possibility, but it is to be borne in mind that the whole question of gluconeogenesis from fatty acids is still in a controversial state (32, 50). Preliminary observations by the method of Behre and Benedict (51) indicate that a diabetic dog with inflammation fails to show in its exudate a significantly higher level of total acetone bodies than is found in the exudate of a non-diabetic dog.

hyperglycemia is intensified only when there is a concomitant inflammation. Pancreatectomy *per se* fails to induce the excessively high blood sugar level which rapidly develops with an accompanying inflammation. Furthermore, a comparative study of exudate and blood sugar in diabetic animals indicates that the exudate sugar tends to be at a higher level than

TABLE 4

A comparison of the exudate and blood sugar concentration in diabetic and non-diabetic dogs

DOG NUMBER	APPROXIMATE DURATION OF INFLAMMATION	BLOOD SUGAR	EXUDATE SUGAR
	Diabetic animals		
	hrs. : mins.	*mgm./100 cc.*	*mgm./100 cc.*
24	7:40	454.5	579.7
25	11:35	241.0	368.3
18	16:35	295.7	419.8
18	41:00	310.7	442.8
8	13:40	482.0	470.7
8	38:00	652.5	869.8
8	63:00	655.7	761.9
6	6:15	311.5	349.9
6	25:15	630.9	629.2
7	33:30	442.9	427.5
Average		447.7	532.0
	Non-diabetic animals		
11	23:00	74.8	73.8
14	24:00	64.2	63.7
12	23:30	55.6	86.2
15	25:30	70.8	71.3
20	6:45	83.9	85.3
20	24:25	111.1	91.2
20	78:40	110.7	46.7
13	10:30	83.8	25.4
13	34:15	61.0	50.9
13	57:35	95.0	40.5
Average		81.1	63.5

that of blood. The results of such observations are compiled in table 4. Animals that received considerable insulin or else which were moribund or at least extremely ill at the time of withdrawal of blood and exudate samples were not included in this series. Studies were also extended to similar specimens from non-diabetic dogs (table 4). The results indicate an average blood sugar in diabetic animals of 447.7 mgm. per 100 cc.

whereas the exudate sugar concentration in these same animals was found to average 532.0 mgm. per cent. By contrast similar comparative studies in a series of 10 control non-diabetic specimens indicate an average blood sugar of 81.1 mgm. per cent, and an average exudate sugar of 63.5 mgm. per cent. These facts show that the exudate sugar of diabetic animals tends to be higher than the corresponding blood sugar, while the reverse seems to exist in non-diabetic dogs. This state of affairs is not wholly surprising inasmuch as the available evidence, described previously, indicates that the excess exudate sugar of diabetic animals is at least in large part derived at the site of inflammation from enhanced local protein breakdown which in turn is readily held in abeyance by insulin administration. As pointed out above, in non-diabetic animals the exudate sugar level tends often to be lower than the corresponding blood sugar. This is most likely referable, as shown by the writer in earlier studies (13), to the higher degree of glycolysis at the site of inflammation than in the blood stream.[4]

It is of significance to point out that the elevated levels of both carbohydrate and protein metabolic products in exudates of diabetic animals are likewise reflected in the circulating blood. The evidence indicates that the heightened concentration of these substances in the blood is evidently referable to absorption from the inflamed area. This in no way contradicts the earlier findings of the writer on the fixation at the site of an acutely inflamed area of various materials (53, 54, 14). The degree of fixation is referable to the intensity of local injury and also to the size of the particle (55, 14). For instance, the writer has demonstrated that proteins are less readily retained than bacteria or graphite particles (56, 57). Miller has essentially substantiated this concept by demonstrating that diffusible substances are rapidly absorbed from an inflamed area (58).

The results of these studies are shown in table 5 and on figure 8. Determinations on blood samples were made before pancreatectomy (column B, table 5), several days after the operation (column A), and when there was an accompanying pleural inflammation (column W.I.). The

[4] There are doubtless several factors which regulate the concentration and distribution of diffusible substances between exudate and blood. Some of these may be briefly enumerated as follows: *a*, diffusibility which tends to equalize the concentration of the materials in both types of fluids; *b*, difference in rate of glycolysis between exudate and blood which thus favors the more rapid degradation of the sugar molecule in exudates (13); *c*, the early appearance of impaired local circulation in an acutely inflamed area in the form of lymphatic blockade (14) might conceivably play a rôle in the speed of equilibration of even diffusible substances between blood and exudate. Notwithstanding these various factors, as well as others, it is interesting to note that the enhanced degree of local gluconeogenesis from proteins in the inflamed area of a diabetic animal seems to transcend the effect of these factors, so that the exudate sugar concentration still tends to be higher than that found in the blood (table 4).

data indicate quite clearly that pancreatectomy *per se*, except for the rise in blood sugar, fails to induce any other very significant changes. On the other hand with the development of a severe inflammatory reaction in the pleural cavity there is not only an enhancement in the blood sugar and lactic acid, but there is also a marked rise in the blood non-protein nitrogen and urea. The amino acid nitrogen likewise shows an appreciable increase in concentration; while the total proteins, with a concomitant pleural inflammation, reveal a slight drop. With pancreatectomy, the concentration of these various carbohydrate and nitrogenous constituents fail to rise in the systemic circulation to the high levels reached when

TABLE 5

Effect of pancreatectomy and of inflammation on some of the carbohydrate and nitrogenous constituents in the circulating blood

DOG NO.	DURATION OF INFLAMMATION	LACTIC ACID			SUGAR			TOTAL PROTEIN			NPN			UREA			AMINO ACID N		
		mgm./100 cc.			mgm./100 cc.			gm./100 cc.			mgm./100 cc.			mgm./100 cc.			mgm./100 cc.		
		B	A	W.I.	B	A	W.I.	B	A	W.I.	B	A	W.I.	B	A	W.I.	B	A	W.I.
	hrs.: mins.																		
1*	7:20	24.8	10.6	30.7	109.1	311.7	497.3	5.9	3.6	4.9	51.9	41.5	86.5	63.0	21.5	53.0	8.3	18.9	8.9
3	15:45	12.9	32.2	71.5	104.2	257.1	522.2	5.1	4.9	4.0	34.3	35.9	83.3	33.5	31.0	124.5	6.9	6.6	10.2
4	24:30	18.8	17.3	12.9	90.1	240.9	412.4	5.4	4.8	4.5	43.0	37.2	49.0				7.4	10.6	8.6
7	33:32	9.9	19.3		104.6	335.3	442.9	4.9	5.7	4.9	26.2	35.1	63.5	18.0	23.0	41.0	7.9	8.0	14.9
19	36:00	19.3	18.4	20.3	98.3	222.2	202.2	5.9	5.5	4.5	32.1	32.0	112.0				4.8	7.4	8.5
8	38:00	16.3	36.1	33.7	73.6	234.0	652.5†	5.9	5.9	5.4	53.6	31.5	106.7				6.8	6.8	9.8
6	25:18	11.1	10.4	23.8	98.2	257.4	630.9	6.6	4.9	3.9	34.0	25.8	70.3	36.5	11.5	51.0	6.0	8.5	10.4
6	48:15			23.8			430.1‡			4.1			109.8			81.5			13.0
Average.		16.16	20.61	30.96	96.87	265.51	473.81	5.67	5.04	4.53	39.3	34.14	85.14	37.75	21.75	70.2	6.87	9.54	10.54

B = Determinations of blood samples withdrawn before pancreatectomy.

A = Determinations of blood samples withdrawn several days following pancreatectomy.

W.I. = Determinations of blood samples withdrawn when there is a concomitant pleural inflammation of varying duration.

* Succumbed 10 min. after removal of blood sample.

† Blood sugar when inflammation was about 44 hours' duration.

‡ Fall in sugar may be referable to starvation.

there is an additional superimposed pleurisy. This would suggest that their elevation is referable to a gradual diffusion or absorption from the site of inflammation.

Similar studies were repeated in a number of non-diabetic animals having an acute inflammation induced likewise by an intrapleural injection of turpentine. The results are shown in table 6 and figure 9. It is clear that neither the carbohydrate nor the nitrogenous metabolism is essentially altered.

The above observations therefore warrant the conclusion that the marked rise in protein catabolism occurring at the site of an acutely in-

flamed area of a depancreatized dog is likewise reflected in the circulating blood. Furthermore, the data indicate that the enhanced proteolysis in inflamed areas of diabetic animals offers a reasonable explanation for local gluconeogenesis from proteins. The surplus glucose formed, in turn, dif-

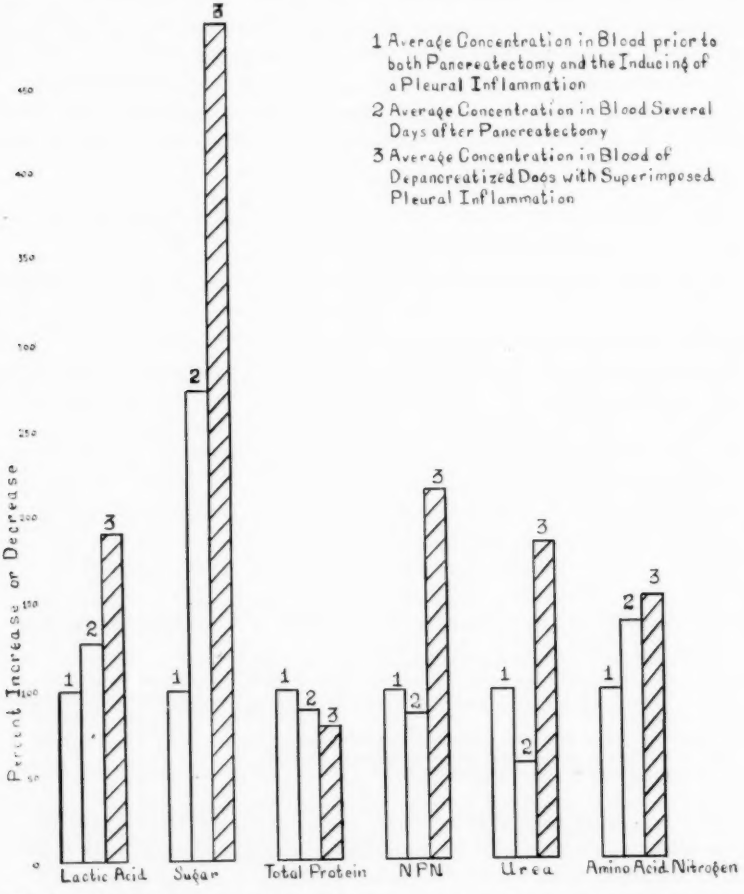

Fig. 8. Effect of inflammation on the concentration of some of the carbohydrate and nitrogenous constituents in the blood of diabetic dogs.

fuses from the area of inflammation into the blood stream thus giving rise to an enhanced state of hyperglycemia.

The increased proteolysis found in diabetic exudates has been shown to be inhibited by repeated administration of insulin (table 3, fig. 6).

In a like manner the parallel increase of these constituents in the circulating blood of a depancreatized dog with an induced pleurisy can readily be repressed by insulin treatment. The results of such observations appear in table 7. The average blood sugar is found to have dropped to an even lower level than that seen prior to the introduction of the inflammatory irritant. It is therefore not surprising to find the NPN and amino acid nitrogen concentration of the blood relatively normal (table 7). These

TABLE 6

Effect of inflammation on some of the carbohydrate and nitrogenous constituents in the circulating blood of non-diabetic dogs

DOG NUMBER	DURATION OF INFLAMMATION	LACTIC ACID		SUGAR		TOTAL PROTEIN		NPN		UREA		AMINO ACID N	
		mgm./100 cc.		mgm./100 cc.		gm./100 cc.		mgm./100 cc.		mgm./100 cc.		mgm./100 cc.	
		B	W.I.	B	W.I.	B	W.I.	B.	W.I.	B	W.I.	B	W.I.
	hr.:mins.												
9	6:25	18.8	17.8	120.3	95.9	6.8	6.2	27.8	13.2	?	15.5	7.2	3.4
	24:50		?		118.1		5.6		35.1		25.0		6.1
	48:05		11.9		109.0		5.8		34.5		35.0		5.6
20	7:00	19.8	19.8	81.7	83.9	6.0	5.4	50.9	33.2			7.0	6.9
	24:45		12.4		111.1		5.2		32.1				7.5
13	10:40	14.9	14.4	112.9	83.8	6.0	5.9	31.4	34.3			7.1	6.0
	34:25		16.3		61.0		5.9		68.4				5.8
	57:45		10.9		95.0		5.8		34.5				5.7
11	23:00	10.9	8.4	71.4	74.8	5.3	4.5	27.8	33.8			5.1	5.1
12	23:10	14.4	10.9	69.8	55.6	6.5	5.9	?	51.0			8.3	5.4
14	24:50	10.9	10.9	63.7	64.2	6.7	5.5	37.2	33.2			4.4	5.2
15	25:05	9.4	8.9	69.3	70.8	7.0	5.4	36.0	44.2			6.8	5.7
26*	6:00		52.5		133.8		4.6		24.1		20.0		6.2
	22:00		22.5		103.0		4.8		22.1		14.0		7.3
Average.....		14.16	16.74	84.16	90.0	6.33	5.46	35.18	35.26		21.9	6.56	5.85

B = Determinations in blood samples withdrawn prior to inducing inflammatory reaction.

W.I. = Determinations in blood samples withdrawn when there was an inflammation of varying duration.

* Partial pancreatectomy prior to inducing the inflammatory reaction.

findings are doubtless referable to a repression by insulin of proteolysis at the site of inflammation. It is interesting to note in this connection that Geelmuyden, recently reviewing the function of insulin, called attention to the fact that "Its most important action is to inhibit the formation of carbohydrate from non-carbohydrates" (59).

The cytological picture of inflammatory exudates in diabetic dogs. With the attending enhanced degree of proteolysis in an area of inflammation

in a diabetic dog, it is conceivable that the cells might manifest detectable signs of protoplasmic injury.

The writer demonstrated several years ago that the cytological picture in an inflamed area is conditioned by the hydrogen ion concentration (52). Polymorphonuclear leukocytes survive a pH above 7.0. At a pH of 6.9

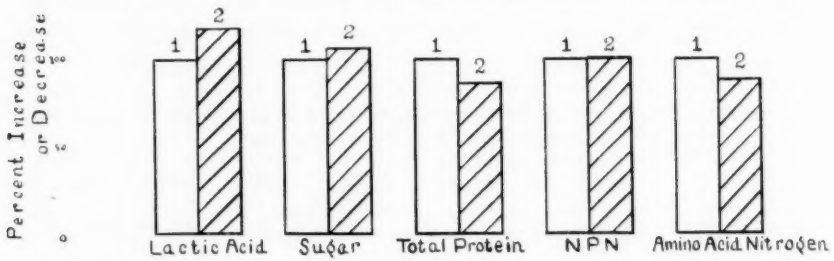

Fig. 9. Effect of inflammation on some of the carbohydrate and nitrogenous constituents in the blood of non-diabetic dogs. 1, average concentration in blood prior to the inducing of a pleural inflammation. 2, average concentration in blood concomitant with an acute pleural inflammation.

TABLE 7

Effect of inflammation on some carbohydrate and nitrogenous constituents of the blood in depancreatized dogs treated with low doses of insulin

DOG NUMBER	DURATION OF INFLAMMATION	BLOOD SUGAR PRIOR TO THE INTRODUCTION OF THE IRRITANT	BLOOD SUGAR SUBSEQUENT TO THE INTRODUCTION OF THE IRRITANT	NPN PRIOR TO THE INTRODUCTION OF THE IRRITANT	NPN SUBSEQUENT TO THE INJECTION OF THE IRRITANT	AMINO ACID NITROGEN PRIOR TO THE INTRODUCTION OF THE IRRITANT	AMINO ACID NITROGEN SUBSEQUENT TO THE INJECTION OF OF THE IRRITANT
	hrs.:mins.	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*
21	6:10	184.2	44.6*	20.6	21.5	4.0	3.7
22	6:35	156.0	71.5*	27.5	14.2	8.6	4.3
	28:00		241.0		37.2		10.7
	47:25		168.1		35.2		12.2
23	7:25	282.6	281.8	?	20.5	?	5.5
	25:05		242.7		29.1		9.2
	47:10		235.3		41.0		10.6
19	11:30	222.2	166.7	32.0	42.0	7.4	5.8
Average.....		211.25	181.46	26.7	30.09	6.67	7.75

* Insulin administered several hours prior to withdrawal of blood sample.

or 6.8 these cells exhibit marked signs of injury and degeneration; but the macrophages appear relatively normal. At a lower pH of 6.5 or thereabouts all forms of leukocytes are injured and frank pus is in evidence. *In vitro* observations further support the view that the cytological picture in an inflamed area is a function of the pH (60). The mechanism of local acidosis was found to be primarily referable to a glycolytic process (13).

The formation of lactic acid from sugar breakdown seems to be mainly responsible for the observed changes in pH and the accompanying cytological shift from polymorphonuclear to mononuclear phagocytes in an inflamed area.

TABLE 8

Effect of inflammation on the pH and the cytological picture of exudates in depancreatized and non-depancreatized animals

DOG NUMBER	DURATION OF INFLAMMATION	pH		CYTOLOGY OF EXUDATE		APPEARANCE OF CELLS IN EXUDATE
		Blood	Exudate	Per cent of polymorphonuclears	Per cent of mononuclear phagocytes	
Experimental depancreatized group						
	hrs.:mins.					
1	7:05	7.42	7.1	Very few cells per field		Swollen, degenerated
3	15:45	7.47*	7.0*	22	78	Degenerated
18	16:35	7.4	7.3	92	8	Normal appearance
4	24:30	7.6	6.95	47	53	Conspicuous vacuolization
6	25:08	7.55	6.9	2	98	Extensive vacuolization; degenerated cells
7	33:12	7.55	7.1	76	24	Swollen and vacuolated
19	36:00	7.55	7.03	88	12	Fairly normal
8	38:00	7.70	7.0	Cells beyond recognition; degenerated, swollen and vacuolated		
Control, non-depancreatized group						
11	23:00	7.47	7.2	90	10	Normal
12	23:30	7.4	7.33	92	8	Normal
14	24:00	7.4	7.28	88	12	Normal
15	25:30	7.38	7.05	82	18	Normal
20	24:25	7:55	7.13	74	26	Normal
13	34:15	7.43	7.07	76	24	Polymorphs are swollen, distorted, vacuolated
16	42:15	7.55	7.4	78	22	Normal
9	24:45	7.57	7.5	82	18	Normal
9	47:50	7.63	7.03	66	34	Polymorphs are swollen, vacuolated and degenerated

* pH measured at 36 to 38°C.; all others at room temperature.

Studies on the cytological picture and the hydrogen ion concentration of exudates in diabetic and non-diabetic dogs were accordingly undertaken. The method utilized to measure the pH has been previously described in detail (52, 13). Cellular smears of exudates were all stained by the usual Wright method.

The results of all observations are summarized in table 8. It is evident

that the pH of exudates in diabetic dogs tends to be somewhat lower than that found in exudates of non-diabetic animals. The differences in pH are fairly well correlated with the respective concentrations of lactic acid in the exudates of both groups of animals (cf. tables 2 and 8).

The cytological picture reveals conspicuous differences. The cells from a diabetic exudate are usually degenerated, swollen or vacuolated. Some cells are unidentifiable owing to the extent of injury. On the other hand, the cells from an exudate of a non-diabetic dog are, as a rule, perfectly normal unless the inflammation is of long duration (i.e., about 2 days). The exudates in non-diabetic animals exhibit in the first twenty-four hours of inflammation a predominance of polymorphonuclear leukocytes. This is in conformation with previous findings (52). On the other hand, exudates from depancreatized animals do not show in the same interval a consistent predominance of polymorphonuclear cells. Histological studies of the pleura in both groups of animals corroborate further the cytological picture of exudates.

The above observations indicate that the enhanced proteolysis in an acutely inflamed area of a diabetic dog not only offers an explanation for active gluconeogenesis, but it also accounts for the increased degree of local tissue damage encountered in diabetes complicated by inflammation.

Discussion. The present results offer a reasonable explanation for the enhanced diabetes in depancreatized dogs with an accompanying acute inflammation. The mechanism, in brief, seems to be primarily referable to an augmented degree of proteolysis at the site of inflammation. The increased protein catabolism seems responsible for the marked degree of local gluconeogenesis. The surplus glucose derived from the degradation of the protein molecule in the injured area gradually diffuses into the systemic circulation, enhancing thus further the elevated blood sugar level.

The findings and the inferences drawn raise several questions. What controlling process in the diabetic organism actually enhances local proteolysis at the site of inflammation? Is gluconeogenesis from proteins in a diabetic animal restricted only to inflammatory foci and to the liver? May it not be true of any area where there is tissue damage, such as, for instance, an arteriosclerotic lesion? To what extent does diabetes interfere with normal protein synthesis, and if so, what possible effects may this have on various immunological phenomena such as antibody formation?

Long (11) recently discussed the possibility that protein catabolism leading to gluconeogenesis may be referable to activity of the anterior pituitary gland. Moreover, the participation of the adrenals, possibly the thyroid, and the liver in carbohydrate and protein metabolic distur-

bances is also not to be overlooked. One of the functions of the adrenal cortex is presumably to regulate, at least to some extent, the level in the circulation of various electrolytes, particularly sodium and potassium (61). It is of interest in this connection that several determinations on the sodium and potassium concentration of exudate and blood of diabetic and non-diabetic dogs have revealed no appreciably significant difference.

As pointed out at the beginning of this paper, in spite of the many obvious similarities, there are some differences between human diabetes and experimental diabetes induced by pancreatectomy. It is therefore of definite importance to extend the present studies to human diabetic patients whose disease is complicated by marked inflammatory processes. It is conceivable that a diabetic individual with only minimal infection may not reveal in the blood stream the striking proteolytic changes detected in dogs having extensive pleural involvement.

CONCLUSIONS

An acute pleural inflammation in a dog fails to alter its blood sugar. On the other hand, the presence of an acute inflammatory reaction in a dog, rendered diabetic by pancreatectomy, induces a rapid and marked elevation in the blood sugar level.

The extent of local proteolysis at the site of an acute inflammation in a diabetic dog is considerably more pronounced than that found in a non-diabetic animal. This is indicated by a lower concentration of the total proteins and a correspondingly higher concentration of urea, non-protein nitrogen and amino acid nitrogen than is encountered in the exudative material of a non-diabetic animal.

The enhanced protein catabolism in the inflamed area of a diabetic animal is correlated with a marked elevation in both exudate sugar and exudate lactic acid. Insulin administration reduces not only the level of sugar and lactic acid in such diabetic exudates; but this substance diminishes as well the degree of local proteolysis. This fact supports the view that gluconeogenesis at the site of an acute inflammation in a diabetic animal originates from proteins through deamination of the molecule.

Enhanced local proteolysis in the inflamed area of a diabetic animal implies increased tissue damage. The cytological picture of a diabetic exudate indicates that polymorphonuclear leukocytes manifest pronounced signs of cellular injury when compared with similar cells derived from a normal exudate.

The biochemical changes encountered in the exudate of a depancreatized dog are similarly reflected in its circulating blood. Besides an elevation in blood sugar level, there is also an increase in the blood concentration of non-protein nitrogen, urea and amino acid nitrogen. Studies after pan-

createctomy or splenectomy, and a comparison of the relative glucose concentrations in exudate and blood, indicate that the raised levels in carbohydrate and nitrogenous constituents of the systemic circulation are neither due to the diabetes *per se* nor to the operative procedure. The elevation in the levels of these substances is referable to an absorption from the area of acute inflammation which complicates the diabetic disorder. Insulin by inhibiting glucose formation in the inflamed area likewise prevents a rise in the blood stream of intermediary products of carbohydrate and protein metabolism.

The available evidences, therefore, support the view that the mechanism of enhanced diabetes with concomitant inflammation might well be referable primarily to an increased local proteolysis in the inflamed area, favoring a combined picture of increased tissue damage with a corresponding elevation in glucose formation; the glucose, in turn, gradually diffuses into the systemic circulation.

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PHOSPHOLIPIDS AS A SOURCE OF ENERGY FOR MOTILITY OF BULL SPERMATOZOA[1]

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In a recent publication (2) it was shown that bull spermatozoa in sugar-free medium maintained motility only in the presence of air. Since deprivation of oxygen is not harmful to spermatozoa in the presence of glucose, we concluded that the spermatozoa were utilizing their intracellular reserves by an oxidative process. The intracellular substance utilized in the oxidative process appeared to be phospholipid. Since the respiration of bull spermatozoa was greatly *decreased* by adding glucose to the suspension medium, the spermatozoa apparently obtain energy preferentially from glycolysis and call upon the oxidative processes *as a source of energy* only when deprived of glycolyzable sugars (2).

While the above-mentioned work was in progress we were also studying the egg yolk-buffer medium (3, 4) in an attempt to determine which constituents of egg yolk were responsible for the effectiveness of this medium in promoting and maintaining an excellent degree of motility for long periods of time. Fractionation studies showed that the protein and neutral fat portions of egg yolk had no beneficial effect in preserving motility of spermatozoa in buffered media. In fact, the neutral fat rendered the spermatozoa immotile. The phospholipid fraction of the egg yolk, however, greatly prolonged the motility of spermatozoa in a buffered medium.

This report deals with experiments designed to study the manner in which the added phospholipids exert their effect on motility. Various preparations of phospholipid were made and their effect on the chemical and physiological behavior of spermatozoa studied.

EXPERIMENTAL. The semen used in the work was obtained from healthy dairy bulls by means of an artificial vagina. The method for motility observations and lactic acid determination and the buffer solution in which the spermatozoa were suspended after centrifuging from the seminal fluid

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have been described (5). The respiration studies were made at 37° with the Barcroft apparatus as in previous work (2). All the data on the oxygen uptake of spermatozoa in the presence of phospholipids are corrected for the autoxidation of the phospholipid itself which was measured in control determinations for each experiment.

Preparation of phospholipids. Fresh egg yolks were extracted twice with 2.5 volumes of acetone. The residue was extracted several times with cold ether and the combined ether extracts evaporated under reduced pressure to a small volume. Ten volumes of acetone were added and the gummy precipitate which formed was again taken up in ether, filtered and reprecipitated. This preparation—*crude egg phospholipids* (I)—was separated by means of ethyl alcohol into *crude lecithin* (II) and *crude cephalin* (III). *Purified lecithin* (IV) was prepared by treating an alcoholic solution of preparation II with alcoholic cadmium chloride and purifying the precipitated cadmium lecithinate according to the method of Levene and Rolf (6). More recently this preparation has been further purified by the procedure of Pangborn (7).

Phospholipids from rat liver (V) were prepared from the ether extract of ground, acetone-extracted rat livers by the procedure used in obtaining preparation I above.

Crude soybean phospholipids (VI) contained 70 per cent acetone insoluble lipids and 30 per cent soybean oil. Soybean "*lecithin*" (VII) and "*cephalin*" (VIII) were obtained from preparation VI by extracting with acetone until oil-free and separation on the basis of their solubilities in ethyl alcohol.[2]

All phospholipid preparations were stored under acetone in the refrigerator to prevent autoxidation. For experimental use the phospholipids were emulsified in buffer solution or 0.9 per cent saline and added at a level of 5 to 10 mgm. per cubic centimeter of the final sperm-buffer suspension.

RESULTS. *Effect of phospholipids on motility.* It is evident from table 1 that phospholipids from egg yolk and other sources were effective in promoting motility. Neutral fat and the mono-ester ethyl laurate were harmful to the spermatozoa. When stored at 10° the spermatozoa maintained their initial motility much longer in the presence of phospholipids than in the presence of added glucose only. From the data in table 2 it is evident that added phospholipid as well as the intracellular reserve of the sperm are utilized by an oxidative process since both are effective in maintaining motility only under aerobic conditions.

Effect of phospholipids on respiration. The rate and duration of oxygen consumption by bull spermatozoa were markedly increased when phospholipids were added to the suspension medium. The results of a typical

[2] We are indebted to Dr. J. L. Gabby of the Glidden Company, Chicago, Illinois, for the soybean phospholipid preparations.

experiment are shown in figure 1. It was found that the increase in respiration of spermatozoa obtained by adding phospholipids depended largely

TABLE 1

Effect of various metabolites on the duration of motility of spermatozoa stored at 10°C.

MEDIUM	METABOLITE ADDED	MOTILITY DURATION
		hours
M/15 Na-K phosphate, pH = 6.75	None	24
	Glucose	78
	Egg lecithin II	151
Modified Ringer-phosphate, pH = 6.75	None	20
	Glucose	50
	Egg phospholipids I	150
	Egg "lecithin" II	150
	Egg "cephalin" III	72
	Liver phospholipids V	150
	Crude soybean phospholipids VI	0
	Soybean "lecithin" VII	120
	Soybean "cephalin" VIII	120
	Egg neutral fat	0
	Ethyl laurate	0

Spermatozoa were centrifuged from the seminal fluid and made up to original semen volume with the buffer solution. Where glucose was added, the final concentration was 0.04 molar. Motility duration indicates the length of time a motility of 1+ or better (5) was maintained.

TABLE 2

Effect of metabolites on sperm motility in air and under nitrogen

MEDIUM, RINGER-PHOSPHATE PLUS	ATMOSPHERE	MOTILITY RATING		
		1 hour	3 hours	5 hours
None	Air	4+	1+	F.M.
	N_2	F.M.	Dead	
0.04 molar Glucose	Air	5+	4+	3+
	N_2	4+	4+	4+
"Lecithin" (II)	Air	4+	3+	3+
	N_2	F.M.	Dead	

Semen was centrifuged, the sperm taken up in 0.9 per cent NaCl, centrifuged again and finally made up to original semen volume with Ringer-phosphate (pH = 6.8). Incubated at 37°C.

on the length of time between the collection of the semen and the beginning of the experiments. This is demonstrated in table 3. Fresh, rapidly respiring spermatozoa showed only a small increase in oxygen consumption

when phospholipids were added while samples which had been held from 2 to 5 hours before beginning the respiration experiments showed considerable increases. As the spermatozoa "age" the intracellular reserves are apparently depleted and the added phospholipid is more rapidly utilized. In ten experiments the addition of phospholipids caused an av-

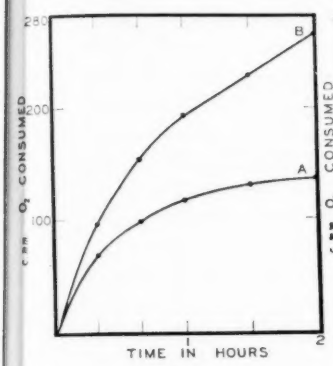

Fig 1

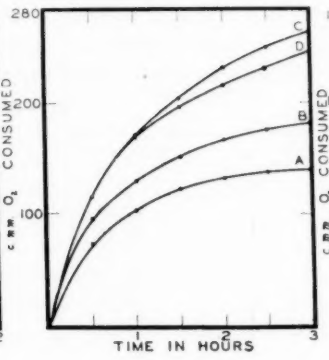

Fig. 2

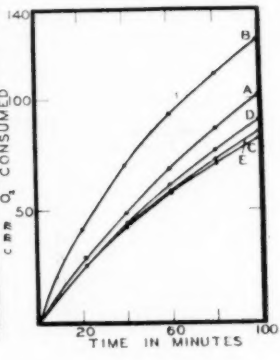

Fig. 3

Fig. 1. Effect of lecithin on oxygen consumption of bull spermatozoa. Semen which had been held at 10° for 4 hours was centrifuged and the spermatozoa were washed by suspending in modified Ringer-phosphate buffer. After centrifuging again the spermatozoa were suspended in modified Ringer-phosphate pH = 6.8. Each Barcroft flask contained one billion spermatozoa in 3 cc. buffer. *A* = endogenous oxygen consumption, *B* = oxygen consumption of spermatozoa in presence of lecithin II.

Fig. 2. Effect of ascorbic acid on the oxygen consumption of bull spermatozoa. Spermatozoa were centrifuged from semen, washed by suspending in 0.9 per cent saline and after centrifuging again were suspended in sufficient modified Ringer-phosphate buffer (pH = 6.8) to give original volume of semen. Eight-hundred million spermatozoa per flask. *A*, no substrate; *B*, with 0.001 molar ascorbic acid; *C*, with lecithin II; *D*, with 0.001 molar ascorbic acid and lecithin II.

Fig. 3. Effect of glucose and lecithin on oxygen consumption of bull spermatozoa. Semen stored 4 hours at 10° was centrifuged and the spermatozoa suspended in sufficient Ringer-phosphate buffer to give original semen volume. One and five-tenth cubic centimeter sperm suspension containing 700 million spermatozoa (= 14.1 mgm. dry cells) per flask. Final volume made up to 3 cc. per flask with 0.9 per cent saline or solution of metabolite in 0.9 per cent saline.

A, no substrate; *B*, with lecithin IV; *C*, with 0.02 molar glucose; *D*, with lecithin IV and 0.02 molar glucose; *E*, with lecithin IV and 0.04 molar glucose.

erage increase in the rate of oxygen consumption of 38 and 46 per cent above the endogenous rate for the first and second hours respectively.

Quastel and Wheatley observed an increase in the rate of fatty acid oxidation by liver slices when ascorbic acid was added (8). This observation together with the demonstration that ascorbic acid promoted the

production of viable, fertile spermatozoa in sterile bulls (9, 10) led us to try the catalytic effect of ascorbic acid on the oxygen consumption of washed spermatozoa (fig. 2). Ascorbic acid itself increased the oxygen uptake of spermatozoa by an amount sufficient to account for its oxidation to dehydroascorbic acid. However, ascorbic acid did not increase the rate of oxygen consumption of spermatozoa in the presence of lecithin.

TABLE 3

Relation of "age" of spermatozoa to oxygen consumption

TIME BETWEEN COLLECTION OF SEMEN SAMPLE AND BEGINNING OF RESPIRATION MEASUREMENT	Q_{O_2}*—FIRST HOUR	
	"Endogenous"	With lecithin VII
hours		
0.5	−9.20	−9.44
0.5	−7.45	−8.05
0.8	−7.04	−8.00
2.0	−6.44	−8.34
3.5	−4.56	−6.90
4.0	−4.27	−7.10
4.0	−4.86	−6.63

* Q_{O_2} = c.mm. O_2/mgm. dry cells/hr.; 37 to 55 million (average 40 million) bull spermatozoa yield 1 mgm. dry matter. Respiration was linear with from 100 million to 1 billion spermatozoa in 3 cc. suspension.

TABLE 4

Effect of phospholipids on lactic acid production by spermatozoa

MEDIUM, RINGER-PHOSPHATE-GLUCOSE PLUS	LACTIC ACID PRODUCED PER CC. SPERM SUSPENSION* IN 3 HOURS	
	Aerobic	Under N_2
	mgm.	*mgm.*
None	0.80	0.83
Egg phospholipids (I)	0.81	0.85

* Sperm concentration 500 million/cc.

Spermatozoa were centrifuged from semen and made up in Ringer-phosphate containing 0.04 molar glucose. Incubated at 37°C.

As shown in figure 3, the addition of lecithin did not increase the oxygen consumption of bull spermatozoa when sufficient glucose was present in the medium. This would indicate that glycolysis occurred in preference to the oxidation of added lecithin just as it does in preference to the oxidation of the intracellular reserves (2).

Effect of phospholipids on glycolysis. Phospholipids did not exert their influence on sperm motility by catalytically increasing the rate of glycolysis either aerobically or anaerobically. As shown in table 4, the amount of

lactic acid produced was the same in the presence of phospholipids as it was without added phospholipids. The fact that lactic acid production was not decreased in the presence of lecithin is further evidence that glycolysis is the preferential source of energy for the bull sperm.

DISCUSSION. The data presented show that phospholipids from egg yolk and other sources were effective in maintaining the motility and oxygen consumption of bull spermatozoa in a sugar-free medium. While it is possible that the added phospholipid exerted its effect by acting as a catalyst or by altering the physical properties of the suspension medium, the more plausible explanation is that it was used as a metabolite. Phospholipids had no effect on motility under anaerobic conditions and did not influence glycolysis under either aerobic or anaerobic conditions. If phospholipids were catalyzing the oxidation of an intracellular metabolite, one would expect an increased utilization and early depletion of this reserve with inactivation of the sperm as a result. On the contrary, lecithin was effective in maintaining the motility of bull spermatozoa for long periods of time. The comparatively poor result obtained with lecithin in previous work (4) was due to the fact that the lecithin preparation used had been obtained from dried egg yolk and was discolored from contact with air. Discolored, partially autoxidized lecithin preparations from sources other than egg yolk have also been found ineffective in promoting motility. A fresh, chemically unchanged preparation was necessary before positive results could be obtained. It is interesting to note that Milovanov found that lecithin increased the life duration of spermatozoa and recommended addition of lecithin to certain semen diluting fluids to protect the lipoid capsule of the sperm cell (11).

It is generally conceded that fats are transported across the cell membranes in the form of phospholipids. It is not surprising then to find that neutral fat or simple fatty acid esters were not utilized by the spermatozoa, for it seems entirely possible that they do not enter the sperm cell.

In metabolic studies on most tissue slices, minced or homogenized tissue suspensions, it is difficult to correlate the respiration or chemical reactions with the function of the tissue. Spermatozoa provide an excellent means of studying the significance of the measured reactions in the maintenance of the function of the cell for the chemical and respiration results can be correlated with motility or also with fertilizing ability. In the work reported here it was shown that the increased oxygen consumption of spermatozoa in the presence of phospholipids was correlated with increased and prolonged motility.

SUMMARY

1. Phospholipids from a variety of sources were effective in maintaining the motility of bull spermatozoa in a sugar-free medium under aerobic

conditions. It was shown that the effect was not due to catalysis of sperm glycolysis.

2. In the presence of phospholipids the rate and duration of oxygen consumption by bull spermatozoa was greatly increased. Ascorbic acid did not catalyze the oxidation of phospholipids by bull spermatozoa.

3. The oxygen consumption of bull spermatozoa *in a medium containing glucose* was not appreciably increased by the addition of lecithin. This, together with the demonstration that lecithin does not alter the rate of glycolysis, is further evidence that bull spermatozoa call upon oxidative processes *as sources of energy for motility* only when deprived of glycolyzable sugars.

4. The similarity in the manner in which added phospholipid and the intracellular reserves are utilized by spermatozoa is in agreement with our previous conclusion (2) that the intracellular reserves of the spermatozoa are phospholipid in nature.

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IODINE FIXATION IN THE THYROID AS INFLUENCED BY THE HYPOPHYSIS AND OTHER FACTORS

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The passage of iodine into the thyroid (1) is easily observed with the help of radio-iodine (2, 3, 4, 5, 6). In our investigation of this problem, the ability of the gland to fix iodine was found to be restricted within definite limits and to be dependent upon the activity of the hypophysis.

METHOD. The use of the isotope I^{128}, with a half-life of 25 minutes, limited the duration of the experiments to two hours. The radio-iodine was prepared by bombarding ethyl iodide with either the neutrons of radon-beryllium or those resulting from the impact of 2000 kv. x-rays on beryllium. The neutrons, producing both radioactive iodine and 2 to 6 mgm. of inactive iodine from the ethyl iodide (4 liters) made it unnecessary to add extra iodine for the purpose of "carrying" the radioactivity. The iodine was extracted as hydroiodic acid by shaking with a concentrated solution of hydrogen sulphide in a separatory funnel. The solution was separated and hydroiodic acid was titrated with sodium hydroxide in presence of methyl orange. After boiling to eliminate the hydrogen sulphide, there remained a solution of sodium (radio-)iodide of known iodine titer, which could be used in animals.

The animals were kept on standard diets (except for the guinea pigs), at 22° (±2°)C. The injections of radio-iodine were performed either in the veins (rat, rabbit) or the heart (guinea pig, mouse). Doses of 0.5 mgm. of iodine were injected per 100 grams of body weight. The animals were sacrificed by section of the carotids 30 minutes after an injection, unless otherwise indicated. For the estimation of the radioactivity in the organs, these were weighed, finely ground and evenly distributed on a rectangular aluminum foil 6 by 2.5 cm. The foil was then fastened lengthwise on a 12 by 5 cm. piece of adhesive tape and the whole covered with cellophane. The adhesive was rolled into a cylinder exactly fitting the Geiger counter in such a way as to set the aluminum in front of the active part of the counter. Less than 100 mgm. of organ was estimated at one time, three measurements being made at 25-minute intervals whenever

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possible. The results were plotted on a logarithmic scale and the best-fitting line was drawn parallel to the slope of the decay of I^{128}. The radio-iodine content was estimated by comparison with the curve furnished by the original radioactive solution, a sample of which had been diluted, put on aluminum, evaporated at 60°C. and measured on the counter in the same way as the organs. The amount of iodine which had been fixed by the organs could thus be calculated. As the justification of chemical and other techniques will be reported separately, it may suffice to state that the accuracy of the determinations was variable, being much reduced in the longer experiments, and that, with the exception of the diiodotyrosine experiment, the limit of error was found to approximate ± 10 per cent.

PRELIMINARY EXPERIMENTS. The fixation of greater amounts of iodine by the thyroid than by other tissues already observed in dogs (1), rabbits (2, 4) and humans (3), is confirmed here in four animal species (table 1). The most regular results were found in the rat thyroid (smallest probable

TABLE 1

Concentration of radio-iodine in the organs thirty minutes after a single injection of Na(radio-) iodide

	CONCENTRATION OF RADIO-I IN THYROID (MGM. PER 100 GM. OF FRESH TISSUE)	CONCENTRATION OF RADIO-I IN LIVER (MGM. PER 100 GM. OF FRESH TISSUE)
Guinea pig	16.2 ± 1.8	0.3
Rat (Wistar)	8.1 ± 0.6	0.5
Mouse (CBA)	7.8 ± 1.5	0.4
Rabbit	14.3 ± 3.2	0.3

Each group was composed of 6 animals.

error), but the greatest fixation of iodine was observed in the thyroid of the guinea pig.[2] The rapidity of the fixation of iodine by the thyroid (1, 2) is illustrated by the experiment reported in figure 1. In addition, these results show that with the dose of iodine used, the concentration of iodine in the thyroid reached a maximum within six minutes after the injection and then remained at a constant level.

In the organs and body fluids not reported in the table, the values were of the same order as in the liver, although very irregular, as indicated by Ariel et al. (5), and without any outstanding concentration of iodine, except in the urine and especially in the gastric juice (6). A chemical separation of the gastric juice of unfed animals, with or without histamine treatment, showed that the iodine behaved like chlorine, since one fraction was in the form of hydroiodic acid, distilling upon gentle heating and precipitating in the distillate after addition of acid silver nitrate; while

[2] In two rabbits, the iodine collection was greater than in any guinea pig.

the other fraction consisted of iodide which remained in the distillation residue wherefrom it could be precipitated out as silver iodide, in nitric medium.

The possibility that other iodized compounds might yield their iodine to the thyroid more readily than the iodides, led to an investigation of the fate of some iodate and diiodotyrosine synthesized from radio-iodine. The use of these compounds presupposed that the iodine atoms present in their molecule could not be exchanged with the iodine atoms of the blood iodide. This possibility was investigated by mixing *in vitro* a solution of radioactive iodide and solutions of more complex iodized compounds as indicated in table 2, in order to see whether or not the radio-iodine would enter the iodate or diiodotyrosine molecule. The results showed the absence of exchange since, within 30 minutes of contact, no radioactivity

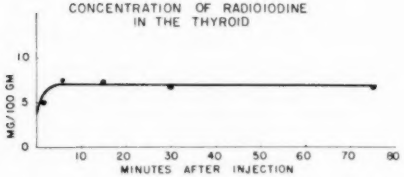

Fig. 1

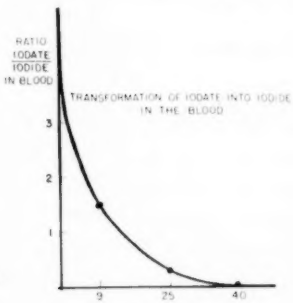

Fig. 2

Fig. 1. Fixation of radio-iodine in the rat thyroid at various intervals after an injection of 0.75 mgm. of radio-iodide. Each dot corresponds to an average of 6 animals.

Fig. 2. Ratio of iodate I to iodide I in the blood at various intervals after an injection of 0.75 mgm. of radio-iodate. Time is indicated in minutes on the abscissa.

that could not be accounted for by experimental errors was exchanged between the iodide and the other compounds examined.

Radioactive iodate, obtained by Baxter and Butler's method (7), was injected intravenously at the dose of 0.75 mgm. of radio-iodine into rats of about 150 grams, in 3 experiments including six rats each. The animals were sacrificed in groups of two at time intervals of 9, 25 and 40 minutes, respectively, after the injection. The amount of radio-iodine in the thyroid averaged 0.9 mgm. and 2.9 mgm. per 100 grams of fresh tissue, respectively, 9 and 25 minutes after the injection; 40 minutes after the injection, the iodine could not be measured any longer, since the radioactivity was then exhausted. The fact that the amount of radio-iodine in the thyroid was small and continued increasing after the ninth minute after injection could be partly explained by an analysis of the fate of the

iodate in the blood stream. This analysis was carried on after the addition of inactive iodide and iodate to the blood to serve as carriers of the suspected radioactive molecules. After trichloracetic treatment of the blood and washing of the precipitate, the subsequent precipitation of the iodate as barium salt and of the iodide as silver salt showed that the radio-iodate decreased while the radio-iodide increased progressively. Therefore in the blood the iodate was transformed into iodide to such an extent that 40 minutes after injection of the iodate only traces of it were still present (fig. 2). The amount of radio-iodine in the thyroid appeared to increase simultaneously with the increase of the amount of iodide in the blood. This suggested that the iodine present in the iodate molecule entered the thyroid only after its transformation into iodide had taken place in the blood.

TABLE 2

Exchanges between iodate or diiodotyrosine or thyroxine and iodine ion of Na radio-I

SUBSTANCES ADDED TO Na RADIO-I	MEDIUM	TOTAL NUMBER OF COUNTS IN NaI AT BEGINNING	DURATION OF CONTACT	PERCENTAGE OF ACTIVITY IN IODATE DIIODOTYROSINE OR THYROXINE AT THE END
			minutes	
Iodate	Water	105,000	30	0.002
Iodate	Boiling water	107,000	30	0.0002
Diiodotyrosine	Water	106,000	30	0.18
Diiodotyrosine	Water	614,000	8	0.05
Diiodotyrosine	Blood	174,000	15	0.06
Thyroxine	Water + 20% of NH_3	406,000	90	0.27
Thyroxine	Water + 20% of NH_3	252,600	30	0.08
Thyroxine	Water + 20% of NH_3	255,000	30	0.50

Similar although less precise results were obtained with radioactive diiodotyrosine, prepared by Oswald's method (8). Relatively high doses of this compound, corresponding to 5 mgm. of iodine per 100 grams of body weight, were given intravenously to each animal in 2 groups of 4 rats each. The time required for the preparation of radioactive diiodotyrosine and its subsequent use in animal experiments precluded exact determinations. With this limitation, the results (table 3) showed, as in the case of the iodate, that the fixation of the radio-iodine in the thyroid was not immediate, and probably followed the rapid transformation of diiodotyrosine into iodide, as observed in blood and urine. In the literature, it has been reported that the transformation of diiodotyrosine into iodide is only partial (9); our different results may be due to the smaller doses of diiodotyrosine used here.

These results were sufficient to warrant the exclusive use of the iodides

in future experiments, since other iodized compounds appeared to be transformed into iodide before their iodine was taken up by the thyroid.

Response of the thyroid to various doses of iodine. In order to test the efficiency of the thyroid gland, doses of iodine varying from 30 to 9000 micrograms were injected into guinea pigs weighing 150 (±20) grams. The results (fig. 3) show that the thyroid fixes increasing amounts of iodine when the doses of iodine are raised. However, if the amount of radio-iodine in the gland is expressed in per cent of the total radio-iodine injected, it can be seen that, with injections of 30, 300 and 9000 micrograms

TABLE 3

Effects of injection of 10 mgm. of radioactive diiodotyrosine

TIME BETWEEN INJECTION AND AUTOPSY	CONCENTRATION OF RADIO-I IN THYROID	PERCENTAGE OF DIIODOTYROSINE IODINE TRANSFORMED INTO IONIZED IODINE	
		Urine	Blood
minutes	*mgm. per 100 gm.*	*per cent*	*per cent*
10–16	<8.5	90	79
30	12.6	91	97.5

of iodine, a thyroid fixation of 1, 0.3 and 0.07 per cent of the total respectively takes place. The smaller the dose of iodine injected, the greater is the proportion of iodine entering the gland. The efficiency of the thyroid is at its best with the smallest doses of iodine. In experiments with smaller or physiological (tracer) doses of iodine, Hertz (10) and Mann (private communication) found that a much higher percentage of iodine was taken up by the gland.

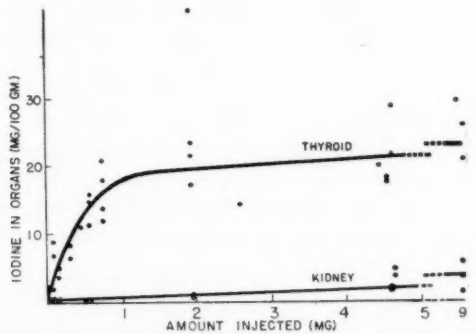

Fig. 3. Fixation of radio-iodine in the guinea-pig thyroid (dots) and kidney (circles) thirty minutes after injection of various doses of radio-iodide.

If the amount of injected iodine was increased beyond 750 micrograms (a dose which happened to be equal to our standard of 0.5 mgm. per 100

grams of body weight), the concentration of 16 mgm. of radio-iodine in the guinea pig thyroid could be increased only slightly even when considerably higher doses were injected (fig. 3). This slow increase of thyroid iodine was similar to what took place in the other organs examined (kidney and stomach wall), where one observed only a slight progressive increase in direct relation to the quantities injected, probably due to a gradual overflooding with iodine. Therefore, with the higher doses, the specific capacity of the thyroid for iodine is exhausted.

However, the curve that indicates this "saturation" of the thyroid (fig. 3) might be simulated by a curve due to exchange equilibria between the iodine atoms of the blood and those of the thyroid. Such an exchange might occur in either of two possible ways. The radio-iodide injected into the blood might exchange with the iodine atoms of diiodotyrosine, or thyroxine, which are the only organic iodized compounds in the thyroid (11). However it has been shown (table 2) that the iodine of these compounds is not exchangeable, at least in 30-minute experiments. As an alternative possibility, the blood radio-iodine could exchange with the small amount of ionized iodine atoms (11) present in the thyroid. This latter possibility was tested by injecting radio-iodide to animals having received a few hours previously some ordinary iodide. If the entrance of iodine into the thyroid were an exchange phenomenon, it should proceed regardless of whether or not a previous injection of iodine had been given. However, when an injection of radio-iodine was given after injections of chemical iodine, only a small fraction of the expected uptake of radio-iodine could be found in the thyroid (table 4, groups 1 and 2). Such results, as already pointed out by Hertz et al. (2), can be explained only by the assumption that some new iodine was added to the gland, with no or little exchange taking place. Indeed Marine and Rogoff using chemical methods have shown the existence of an addition of iodine to the gland after iodide injection, but their experiment did not rule out the possibility of a simultaneous exchange (1). It may be concluded that, after an addition of about 16 mgm. of iodine per 100 grams of thyroid (guinea pig), this gland reaches a state of saturation. In a saturated thyroid, it is not possible to produce an important increase of the iodine concentration by the injection of either large (fig. 3) or repeated (table 4, groups 1 and 2) doses of iodine.

Exchanges being ruled out, at least for the most part, the saturation could be due to a chemical process, since the iodine entering the thyroid could react with free chemical linkages, which, being then saturated, would not be able to fix additional iodine. In order to decide whether the radio-iodine in the thyroid had undergone some chemical transformation or was still in the form of iodide, chemical analysis of the gland was performed thirty minutes after a standard injection of radio-iodine into guinea pigs.

The tissue was ground and extracted with hot alcohol; and after filtration and evaporation of the filtrate, the residue containing all the radioactivity was dissolved in alkaline solution, precipitated with trichloracetic acid and filtered. About 90 per cent of the total radioactivity was in the filtrate, where it could be precipitated as iodide with acid silver nitrate, the rest of the radioactivity being in the trichloracetic precipitate with the thyroglobulin. Hot water extraction, dialysis and electrolysis in acid medium on a copper plate, also indicated that at least 90 per cent of the radio-iodine was still present in the thyroid as iodide, while it seemed as though a fraction less than 10 per cent had been incorporated into the molecule of thyroglobulin. Therefore the entrance of iodine into the thyroid was not the direct consequence of a chemical reaction; its accumulation

TABLE 4

Fixation of radio-iodine in thyroid of animals having received previous injections of inactive iodine

EXPERIMENT NUMBER	AMOUNTS OF IODINE INJECTED BEFORE RADIO-ACTIVE IODINE	INTERVAL BETWEEN LAST INJECTION OF IODINE AND RADIO-ACTIVE IODINE	AMOUNT OF FIXED RADIO-ACTIVE IODINE (IN MGM. PER 100 GM. OF FRESH TISSUE)	
			Thyroid	Liver
1	0	(controls)	6.7	0.4
	1 mgm. daily for 8–10 days	5 hours	1.8	0.3
2	0	(controls)	8.4	0.2
	1 mgm. daily for 8–10 days	8½ hours	1.1	0.1
3	0	(controls)	11.8	0.4
	1 mgm. daily for 8–10 days	48 hours	3.2	0.3
4	0	(controls)	8.7	0.3
	1 mgm. daily for 8–10 days	72 hours	6.8	0.4

in this instance would seem to be more in the nature of a process of absorption.

Although the short life of the radio-iodine prevented an analysis of its transformation in the thyroid over a period longer than 30 minutes, an indirect method gave some information as to the ultimate fate of the fixed iodine. Since a thyroid lost its ability to fix iodine when saturated, an experiment was designed to determine the time required for the restoration of a normal iodine fixation. A preliminary experiment, performed a week after saturation of the thyroid with inactive iodine, showed that the gland had at that time completely recovered its ability to fix iodine. Eight groups of five rats weighing about 150 grams were then subdivided into 4 control groups and 4 groups receiving 1 mgm. of iodine in the form of

iodide daily for 8 to 10 days, this dose being insufficient for a reduction of the thyroid activity of the animals as judged from the histology of the gland. A single injection of 0.75 mgm. of radio-iodine as NaI was then administered to the control animals, while the treated animals were given the same dose at various intervals after their last injection of inactive iodide (table 4). The results indicated that three days after the last injection of inactive iodide, the iodine-fixing capacity of the thyroid had almost returned to normal (fig. 4). The time required for the restoration of the ability of the gland to fix iodine may indicate that the amount of iodine necessary to saturate the gland was utilized in a little more than three days.

Rôle of the hypophysis. Hypothyroidism was produced in the rat by hypophysectomies performed by A. Chamorro (4). This operation re-

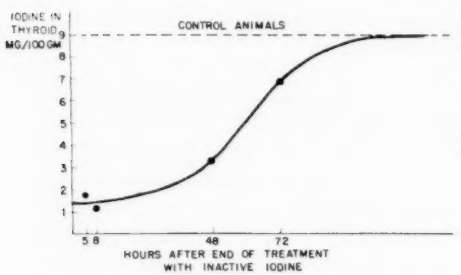

Fig. 4

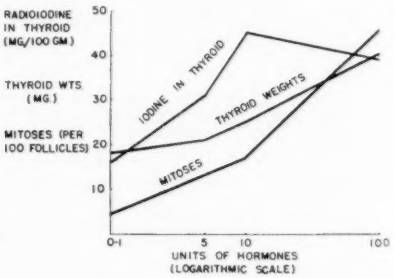

Fig. 5

Fig. 4. Fixation of radio-iodine in the rat thyroid when 0.75 mgm. of radio-iodide was injected at various intervals after saturation of the thyroid.

Fig. 5. Effect of injection of 5, 10 and 100 units of thyrotropic hormone on the weight, mitotic activity and iodine-fixing ability of the guinea-pig thyroid.

sulted in a decrease of the ability of the thyroid to fix iodine (table 5). The decrease was not very sudden, however, since 5 days after removal of the hypophysis, the fixation of iodine by the thyroid was only slightly reduced. At 116 days after the operation, the concentration of iodine in the thyroid was very small, although still greater than that of other organs, such as the liver.

The decrease in the concentration of radio-iodine after hypophysectomy could be due to a relative decrease of active thyroid tissue, since the operation produces an accumulation of colloid with a reduction in the size and probably in the number of the cells. Another alternative was that the remaining cells had lost their efficiency in fixing iodine. The relative amounts of cells and colloid were estimated on histological sections of the thyroids in two animals operated 20 days previously and their two controls, as also in two operated 116 days previously and their two controls. Cam-

era lucida drawings of 4 wide (Reichert) fields on thyroid sections at a magnification of 150 × were performed on each animal. By cutting off and weighing the cells and colloid of the drawing, it was found that the average ratio of colloid to cells had increased from 0.69 to 3.13 in the twenty days following hypophysectomy and from 0.62 to 4.7 in the four months following this operation. Simple equations deducted from these figures showed that, 20 and 116 days after the operation, the relative amount of cells had decreased by factors of 0.41 and 0.28 respectively. Since the radio-iodine concentration in the thyroid had decreased by factors of 0.42 and 0.11 respectively in these two groups (table 5, last column), it can be seen that this reduction in the iodine uptake was comparable to the reduction in the amount of thyroid cells.[3] If, therefore, the thyroid

TABLE 5

Fixation of iodine by rat thyroid after hypophysectomy

NUMBER OF DAYS AFTER OPERATION	NUMBER OF ANIMALS	AVERAGE BODY WEIGHT		AVERAGE WEIGHT OF THYROID	PER CENT OF INJECTED RADIO-ACTIVITY IN THYROID	CONCENTRATION OF RADIO-IODINE (MGM. PER 100 GM. OF FRESH MATERIAL)		RATIO OF THYROID RADIO-I CONCENTRATION: TREATED / CONTROLS
		Before operation	At autopsy			Thyroid	Liver	
		grams	*grams*	*mgm.*				
0	5	156	157	12	0.12	7.2	0.3	
6	5	142	129.3	9	0.08	6.8	0.4	*0.94*
0	1	82	87	9	0.10	8.7		
9	1	82	56	8	0.03	4.3		*0.49*
0	2		136	13	0.15	9.1	0.4	
20	2		60	7	0.03	3.8	0.6	*0.42*
0	5	131	196	18	0.24	11.7	0.5	
116	7	114	109	11	0.02	1.25	0.4	*0.11*

of the hypophysectomized animal fixed less iodine, it was due mostly to the small amount of thyroid tissue present in these thyroids, although a slight decrease in the efficiency of the remaining cells was not excluded, especially four months after the operation.

Hyperthyroidism, obtained through injections of a highly purified Schering thyrotropic hormone into guinea pigs (8 injections in 4 days), produced a marked increase in iodine uptake by the thyroid (table 6). The increase in the concentration of radio-iodine could be due either to the relative increase of active thyroid cells following the departure of colloid, or to an increase in the efficiency of the cells, or to both factors. In

[3] It was assumed for these calculations that the changes in the connective tissue were negligible. This implication involved only a small error.

three control and three guinea pigs treated with 10 units of thyrotropic hormones, the ratio of colloid to cells was estimated as above. The decrease of these ratios from 0.80 to 0.43 under the influence of the injections implied that the relative amount of colloid had decreased by a factor of 1.48 and the proportion of cells had increased by a factor of 1.26. On the other hand the concentration of radio-iodine in the thyroid increased, after injection of 10 units of hormone, by a factor always superior to 2, reaching 4.2 in one group (table 6, last column). The increase in the "cell con-

TABLE 6

Fixation of iodine by guinea pig thyroid after thyreotropic hormone

NUMBER OF EXPERIMENT	UNITS OF HORMONE IN 4 DAYS	NUMBER OF ANIMALS	AVERAGE THYROID WEIGHT	PER CENT RADIO-ACTIVITY IN THYROID	CONCENTRATION OF RADIO-IODINE (MGM. PER 100 GM. OF FRESH MATERIAL		RATIO OF THYROID RADIO-I CONCENTRATION: TREATED / CONTROLS
					Thyroid	Liver	
			mgm.				
1	0	5	21	0.3	13.5	0.2	
	10–12	6	39	2.1	43.3	0.1	3.2
2	0	3	20	0.3	11.8	0.4	
	10	3	21	1.3	51.4	0.4	4.3
	100	3	36	2.4	49.6	0.5	4.2
3	0	3	21	0.5	18.0	0.3	
	10	2	26	1.5	43.2	0.2	2.4
	100	3	44	1.8	35.9	0.3	2
4	0	2	21	0.4	14.3	0.1	
	5	3	23	0.9	30.0	0.2	2.1
	10	2	25	1.3	37.6	0.2	2.6
	100	2	42	2.1	35.3	0.1	2.4
5	0	2	11	0.3	21.1	0.1	
	2	3	19	0.7	26.9	0.1	2.0
	5	2	20	0.9	34.4	0.1	2.8
	100	2	40	1.6	32.4	0.1	2.4

centration" could account for only a small part of the increase in radio-iodine concentration.[3] Therefore the cells must have been functioning more efficiently when under the influence of thyrotropic hormone. In other words, the thyrotropic hormone had increased the capacity of the thyroid gland for iodine, partly by reducing the amount of colloid and thus increasing the cellular content, but mostly by increasing the activity of the cells.

The previous analysis of the effects of thyrotropic hormone was based upon results in animals receiving injections of 10 units of this hormone

in 4 days. Increasing the amount of hormone to 100 units, however, did not produce further concentration of iodine in the thyroid (table 6). The understanding of these results was facilitated by plotting them on a logarithmic scale (fig. 5), along with the average weights of the glands and the average number of colchicine mitoses per 100 follicles. The mitoses were counted on 5-micron sections in 300 thyroid follicles in each of 18 guinea pigs divided into groups of 6, the groups receiving 0, 10 and 100 units of thyrotropic hormone respectively over a period of 4 days. The animals were given colchicine 9 hours before autopsy (12). Increasing the dose of thyrotropic hormone from 10 to 100 units produced a considerable growth of the thyroid as shown by the weight and mitosis curves, but the radio-iodine concentration decreased. The radio-iodine concentration was smaller with 100 units than with 10 units of thyrotropic hormone in each case (table 6, 5th column), thereby indicating not only that the efficiency of the thyroid tissue could not be increased over the limit reached with 10 units of hormone, but that the newly formed thyroid tissue, testified to by the mitoses and weight increase, could not be stimulated as effectively as the original thyroid tissue.[4] It is a frequent occurrence in biology that the function of rapidly growing tissue is somewhat hampered by the growth itself, as shown for instance by Doljanski (13).

Discussion. Intravascular administration of sodium iodide was followed by a deposition of iodide as such in the thyroid gland. The amount of iodide that could be collected by the gland was limited in the rat to about 10 mgm. per 100 grams of fresh tissue and in the guinea pig to nearly 20 mgm. per 100 grams of fresh tissue. These values could be but slightly increased by injecting larger or repeated doses of iodine, and therefore indicated the extent of the capacity of the thyroid for ionized iodine.

Some of the results (fig. 4) suggested that the amount of iodide necessary for saturation of the thyroid gland was utilized in a little more than 3 days. The amount used up in 3 days could be roughly estimated by the difference between the figures obtained for the radio-iodine concentration in the thyroid of the treated animals in groups 1 and 4 (table 4), that is to say, 5 mgm. per 100 grams of fresh tissue. The rapidity with which this iodine stored in the thyroid may be utilized was unexpected, and points to the necessity of a regular iodine intake.

When by hypophysectomy the source of thyrotropic hormone was eliminated, the radio-iodine and the cellular content of the thyroid decreased in the same proportion. It thus seems that, after the operation, the remaining cells, in spite of their atrophy, can fix as much iodine as an equal cytoplasmic volume of normal cells. Therefore the operation does not affect the specific iodine-fixing ability of the thyroid tissue, although a slight decrease of this activity was not excluded by the present data.

[4] There is a possibility that higher doses of iodine were required in this case.

Injections of thyrotropic hormone increased the iodine fixation in the thyroid by double mechanism. An increased proportion of thyroid cells was produced and the efficiency of these cells was increased. The latter result shows the possibility for thyrotropic hormone to influence the efficiency of the cells. However, when no extra dose of this hormone is given, the level of activity of the thyroid cell is quite stable and is not much influenced by the suppression of thyrotropic hormone, as afforded by pituitary removal.[5]

CONCLUSIONS

1. The specific ability of the thyroid to fix iodine has been confirmed.
2. Experiments with radioactive iodate and diiodotyrosine suggest that only ionized iodine can be withdrawn from the blood stream by the thyroid gland.
3. The smaller the dose of iodine administered, the more efficiently it is fixed by the thyroid.
4. When doses of half a milligram or more of iodine per 100 grams of body weight are injected intravascularly, the thyroid becomes saturated with iodine and is no longer able to fix iodine selectively. In other words, the capacity of the thyroid for iodine is limited.
5. Exchange phenomena or chemical transformations play little or no rôle in the penetration of ionized iodine into the thyroid.
6. After being saturated with iodine, the thyroid recovers its normal ability to fix iodine in a little more than 3 days.
7. The extent of the decrease in iodine fixation after hypophysectomy and of its increase after injection of thyrotropic extract indicate that the hypophysis does not influence the specificity of the thyroid activity, but regulates the fixation of iodine by controlling to some extent the amount and the efficiency of the thyroid tissue.

We wish to acknowledge our debt to Prof. F. Joliot-Curie for having made possible the use of radio-iodine and for his numerous suggestions in the physical, chemical and biological fields. We also express our appreciation to Dr. K. E. Mason and Dr. S. L. Warren for the facilities afforded during the writing of the manuscript, and to Dr. V. E. Emmel for his assistance in its preparation.

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METHODS FOR THE COLLECTION OF FLUID FROM SINGLE GLOMERULI AND TUBULES OF THE MAMMALIAN KIDNEY[1]

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In 1921 Wearn and Richards (1) demonstrated the possibility of collecting fluid from single glomerular capsules in the living frog's kidney. In the 20 years which have succeeded this demonstration, methods have been gradually developed for the collection of fluid from single tubules of the amphibian kidney and for the quantitative analysis of the minute amounts of fluid obtained. The body of evidence accumulated by the application of these techniques has lent clear and decisive support to the filtration-reabsorption theory of urine formation (2). Glomerular fluid has been shown to have the composition of an ultrafiltrate of blood plasma in the eleven respects in which it has been examined. Reducing substances, chlorides and fluid have been shown to be reabsorbed from this filtrate as it passes through the tubules, and the *locus* of these reabsorptive processes has been established. The extension of this type of experimentation from the amphibian to the mammalian kidney has seemed desirable, not alone because the truth of the filtration-reabsorption theory could again be subjected to thorough examination, but also because the functional and anatomical differences between kidneys of the two types made it certain that new information would be disclosed. Progress has been made in this extension and the present paper describes methods which have proven successful in collecting fluid from single glomeruli and tubules of the mammalian kidney.

Preparation for visualization of the kidney surface. A majority of the experiments have been performed on guinea pigs and rats. In both species, a unilateral (right) nephrectomy was done from 5 to 43 days before the experiment in order to produce enlargement of the nephrons in the remaining kidney. This operation was performed on 89 guinea pigs and 56

[1] The expenses of this work have been defrayed in large part from a grant by the Commonwealth Fund of New York. A preliminary report of the investigation was made to the American Physiological Society in April, 1941 (This Journal **133:** 480, 1941).

rats under aseptic precautions and with ether anesthesia. If it is assumed that the weight of the two kidneys was originally the same, hypertrophy of the remaining kidney was apparent by the fifth day post-operative, became maximum during the third week when it averaged 71 per cent in the rats and 62 per cent in the pigs, and remained stationary after that time. The subsequent procedures differed somewhat in the two species and must be described separately.

Guinea pigs. The guinea pigs, adult females weighing between 400 and 600 grams, were injected intraperitoneally with 0.45 cc. (29 mgm.) of sodium pentobarbital. Ether was given when necessary during the preparation. Sodium barbital, chloralosane and urethane were substituted for pentobarbital in a few experiments. The animal holder was a copper plate embedded in a sheet of cork and heated from beneath by a 15-watt bulb; the animal's body lay on the plate, and the cork provided attachment for the retractors and for the pins which restrained limbs and head. The trachea was isolated for the subsequent insertion of an 18-gauge needle, permitting insufflation with oxygen, and the right jugular vein was cannulated and connected with a burette containing a solution of 0.9 per cent sodium chloride or 10 per cent sucrose. Blood pressure was not usually measured. After the abdomen was opened by a mid-line incision, the entire gastro-intestinal tract was removed following successive ligation of the coeliaco-mesenteric axis, inferior mesenteric artery, esophagus, and gastro-hepatic omentum. The urinary bladder was emptied. Fat and peritoneum were removed from the lower pole of the kidney surface. The renal capsule was usually left *in situ* since it did not interfere with visibility or puncture of the surface units and since, if it were removed, the visible blood vessels became dilated, the surface covered with a layer of protein-rich fluid, and puncture was more apt to result in tears of the tubule walls or hemorrhage from the vessels which border them. The abdominal wall was retracted and raised, and the abdominal cavity filled with liquid petrolatum (light), warmed to body temperature, which covered the kidney to a depth of about 5 mm. This oil prevented the kidney from drying, aided in the diffusion of light, and could be readily distinguished from the watery fluid that originated in the nephrons. Its temperature remained between 35° and 39°C. during the experiment.

The surface of the kidney was illuminated by a 300-watt bulb focused on the butt end of a lucite rod 12 inches in length, 0.5 inch in diameter and ground down at one end to a bevelled tip 0.08 inch in diameter. When this tip was brought into contact with the oil by a micromanipulator, a considerable portion of the kidney surface was brilliantly illuminated but pulsatile and respiratory excursions made it impossible to visualize details of structure. Movements of the former type were diminished by exerting downward and lateral pressure on the upper pole of the kidney with a glass

rod, terminating in a disc one-half inch in diameter and shaped to fit the kidney surface; if the movements were still too gross, a small area could be immobilized by exerting downward pressure with the lucite rod at a point close to the glass disc. The movements due to respiration were particularly troublesome in the guinea pig and after attempts to inactivate the diaphragm and to fix or support the kidney proved unsuccessful, recourse was had to paralyzing the respiratory muscles with curare. One milligram of curare,[2] injected subcutaneously, abolished movements within

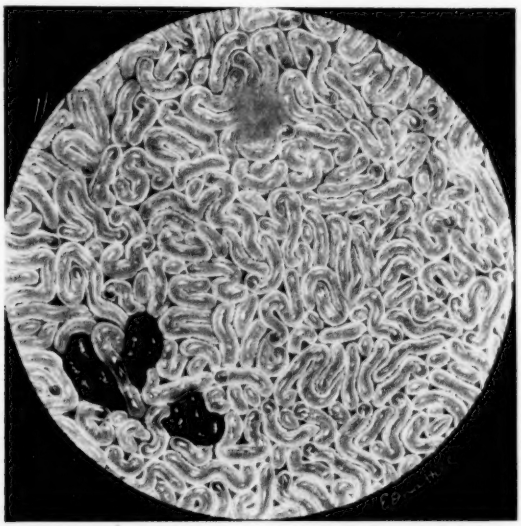


Fig. 1. Appearance of the ventral surface of a guinea pig's kidney when observed by the methods described in the text. India ink has been injected into a single tubule segment and has filled the 3 coils of a proximal convolution shown at 7 o'clock. The rounded interruption of the tubule pattern at 12 o'clock is a glomerulus. Photograph of a drawing made from life by Miss Edna Hill through the courtesy of the Harrison Department of Surgical Research, University of Pennsylvania Medical School. Magnification, approximately 50×.

ten minutes and oxygenation was maintained by intra-tracheal insufflation with 100 per cent oxygen. Under these circumstances the kidney was completely immobile and, examined with a binocular microscope at 85 magnifications, presented the appearance illustrated by the drawing of figure 1.

During the first hour after the preparation was completed, the kidney

[2] The specimen employed was supplied by the courtesy of Dr. Hans Molitor of the Merck Institute for Therapeutic Research.

continued to form urine at the rate of about 1 cc. per hour and retained its ability to reabsorb glucose and fluid, for the urine was practically free of fermentable reducing substances and contained exogenous creatinine in concentrations averaging 47 times that in blood plasma. Urine and blood specimens were collected at the beginning and end of each experiment by direct puncture of the bladder and vena cava with glass capillary pipettes containing, in the latter instance, an anticoagulant.

The chief disadvantage of the guinea pig preparation was the brevity of the period during which normal kidney function persisted. Within 1.5 hours after respiration was arrested the surface tubules were no longer distended with fluid, their epithelial lining became white and more readily visible, and the surface blood vessels showed brief cycles of contraction and relaxation (about 5 and 15 sec. respectively) which were apparently due to concurrent changes in some major renal vessel since they were accompanied by gross alterations in kidney size. When the blood pressure was measured at this time it was usually found to have fallen markedly. A second disadvantage was the considerable decrease in plasma concentrations of glucose and exogenous creatinine which occurred during the period of observation. This decrease, in the case of glucose, was especially marked after phlorhizin administration and might have been anticipated from the exclusion of the hepatic circulation but, in the case of creatinine, its mechanism was less clear.

Rats. The rats, adult males weighing between 300 and 400 grams, were starved for 18 hours preceding the experiment. They were anesthetized by the esophageal injection of 2.0 grams of urethane per kgm. in 10 cc. of tap water. The trachea was isolated, the jugular vein cannulated, and the abdomen incised in the mid-line as in the guinea pigs. The viscera were not removed but simply withdrawn from the vicinity of the kidney by retractors. The bladder was emptied, the abdominal wall supported, the abdominal cavity filled with oil and the kidney illuminated as in the guinea pigs. Blood specimens were taken by cutting the tip of the animal's tail. It proved unnecessary to arrest respiratory movements; if they were troublesome, the intravenous injection of 100 mgm. of sodium barbital usually diminished them to a point where they, as well as the pulsatile movements, could be controlled with pressure by the glass disc and lucite rod. This preparation was superior to that of the guinea pig in many respects. The entire operation could be completed in 15 minutes, the kidney was not touched, the concentrations of glucose and exogenous creatinine in blood plasma remained relatively constant, blood could be obtained at any time during the experiment, and the animal's condition continued good for 3 hours or more. A single advantage was retained by the guinea pig. In that species alone have we been able to see functioning glomeruli.

Collection of fluid from glomeruli. It seemed essential to the success of this investigation that we obtain at least a few specimens of glomerular fluid. Persistent attempts were therefore made to visualize glomeruli on the kidney surface of a variety of animals. The earlier of these attempts[3] amply confirmed Bowman's statement, made in 1842, that "the Malpighian Bodies are rarely if every visible quite on the surface of the kidney" (4). The topographical relationship of typical superficial nephrons to the kidney surface is shown in figures 6 to 10 (insets) where the glomeruli are seen to be covered by several layers of proximal convolutions. Even in the skunk, where glomeruli often lie within 0.2 mm. of the kidney surface, they are invisible by our methods of examination. In the guinea pig, however, we have had some measure of success.

In about one out of four guinea pigs, when the entire anterior surface of the kidney was examined, one or more round objects were seen which proved to be glomeruli. In the rare instances when these glomeruli lay

[3] The kidney surface of the following animals has been examined during life in an attempt to visualize glomeruli: bats (2), adult cat (1), cat aged one month (1), ferret (1), adult white mouse (1), new-born white mice (4), deer-mouse (1), muskrat (1), adult opossums (25), pouch opossums from 2 to 7 weeks after entering the pouch (4), rabbits (8), adult rats (56), new-born rat (1), skunks (7), and gray squirrel (1). No glomeruli were observed in any of these animals except in one opossum kidney within an area of focal nephritis and in one skunk kidney which was examined postmortem. Large round objects were seen in the younger pouch opossums but histological examination did not suggest that these were functioning glomeruli. We varied our method of illumination by placing the lucite rod beneath the kidney, as is possible in the bat, by thrusting it into the kidney substance and into the cysts which occasionally occur in the opossum kidney; no one of these alterations made the glomeruli visible though, in many of the animals examined, some were demonstrated to lie within a millimeter of the kidney surface. Two further variations in technique were employed in skunks, opossums, and rabbits. In the first we attempted to color the glomeruli so that their visibility might be improved; the abdominal aorta below the renal arteries being ligated and the coeliac axis cannulated, a 0.1 per cent solution of Janus Green B was injected during momentary arrest of the renal circulation; although the glomeruli proved to be well colored when the kidneys were sectioned, they were not visible from the kidney surface. Finally it was determined to expose the sub-surface glomeruli by removing a slice of kidney tissue. Either at a preliminary operation or at the time of the experiment, a thin section was removed from the kidney surface by a razor, hemorrhage being arrested by momentary clamping of the renal artery and by the application of the cut edge of a piece of skeletal muscle. A large number of glomeruli became readily visible but we were unable to collect any fluid from them and they did not appear to be functionally active. The glomerular capillaries were dilated, the corpuscles within them stagnant, and this appearance did not change when the renal blood flow was interrupted or when adrenalin or sucrose was injected intravenously. Moreover, the cut surface of the kidney was covered with a layer of exudate or tissue fluid. The technique was discarded as being grossly unphysiological, a decision which may have been premature since Ellinger (3) has described the appearance of fluorescent dyes in sub-surface glomeruli of rats exposed by an identical procedure.

completely on the kidney surface, the individual capillaries could be identified and blood could be seen flowing through them. We have only seen six such glomeruli in nearly 100 animals. There was no visible clear capsular space about them, as is the case in amphibia, and when they were punctured by a quartz pipette a capillary was torn with consequent hemorrhage from the tuft followed by permanent stasis. On one occasion puncture was accomplished without hemorrhage but apparently resulted in contraction of the afferent vessel, for all of the capillaries emptied themselves of blood and remained contracted. No one of these glomeruli showed intermittence of blood flow, but only six were observed and no one of them for over 5 minutes. The majority of glomeruli seen were less clearly visualized but proved more suitable for our purpose. They appeared as rounded reddish objects, either below the kidney surface and covered by a single layer of tubules, or as a structureless interruption in the pattern of surface tubules (fig. 1). These glomeruli could usually be punctured without hemorrhage and, in 7 experiments, sufficient fluid has been collected from them for analysis. The puncture was performed with a quartz pipette, about 7μ in internal diameter at its tip, attached to a glass rod and micromanipulator in the fashion which has been described (1). The pipette was filled with mercury except at its extreme tip, where a small quantity of a light oil colored with Scharlach-R had been introduced. When the glomerular capsule had been penetrated, the oil was injected; at first it filled and distended the capsular space and then flowed on into the proximal tubule which might or might not reach the kidney surface. As soon as a millimeter or so of the proximal tubule had been filled with oil, the oil remaining in the capsular space was evacuated into the pipette and the collection of glomerular fluid commenced against a slight positive pressure in the collecting system. The oil served the double purpose of identifying the punctured object as a glomerulus and, by its continued presence in the proximal tubule, gave assurance that the collected fluid was derived from the glomerulus rather than from any more distal portion of the nephron. The collection was continued until sufficient fluid had been secured for analysis or until it was terminated by some accident. In the 7 completed experiments the average amount of fluid obtained was 0.24 c.mm. Subsequent dissection of the nephron, by the methods to be described, proved whether or not any tubule bordering the glomerulus had been accidentally punctured during the experiment.

Two matters should be mentioned since they may detract from the significance of these experiments. Glomerular fluid, collected from amphibia, proved to be free[4] of protein (5). Of the 6 specimens of this series which

[4] The method cannot consistently detect concentrations below 0.03 per cent. The statement, in this paragraph, that a specimen contained no protein should therefore be taken to mean that it contained less than 0.03 per cent.

were similarly tested only two were negative, two contained between 0.15 and 0.20 per cent, and two contained amounts estimated at 0.80 per cent. This need not mean that the normal mammalian glomerulus excretes any considerable amount of protein, for the great majority of tubule fluid specimens and two from within 1 mm. of the glomerulus were protein-free. But it does suggest that glomerular capillaries leak protein readily even in the absence of gross damage, and that the glomeruli in question were affected either by the act of puncture or by the injection of oil. In the second place, the rate at which fluid was collected was about 30 per cent lower than would be anticipated on the basis of calculations made from creatinine clearances (6), the average figure in the 7 experiments being 0.7 c.mm. per glomerulus per hour. While the collections may have been incomplete due to leakage of fluid through tears in the glomerular capsules, these slow rates may suggest that the surface glomeruli, after puncture at any rate, were less active than those which lay deeper in the kidney substance.

The collections of glomerular fluid therefore were neither very numerous nor wholly free from objection. Fortunately the conclusions to which they led received support from the more numerous and satisfactory experiments upon proximal tubules.

Collection of fluid from proximal convolutions. As will be understood from an examination of figures 5 to 10, the vast majority of the tubule segments appearing on the kidney surface proved to be portions of the proximal convolutions. In a good preparation they were distended with fluid and presented the appearance illustrated in figure 1. Each segment looked precisely like its neighbor and it was impossible to distinguish anatomical portions of the proximal tubule from each other or from the distal tubule by simple observation. Two juxtaposed segments were not necessarily portions of the same nephron and their relationship to each other could only be shown by such an intratubular injection as is illustrated in figure 1 where india-ink was used. The tubules were larger in adults than in young and increased in size after unilateral nephrectomy. Of the animals examined, they were largest in the opossum and skunk. In the guinea pig the continuous segments on the surface were rather longer than in the rat. An active circulation could be seen in the blood capillaries which bordered each segment and, on occasion, crossed over a tubule. If the circulation failed, or if the renal artery was clamped, the lumina of the tubules collapsed and their walls became more apparent; under these circumstances some tubules appeared white while others showed brownish granulations but these differences did not prove to be characteristic of any particular portion of the nephron. If the tubules were collapsed when first observed, they could be promptly distended by the intravenous injection of 1.0 cc. of a 10 per cent sucrose solution. The effect was much more marked than that pro-

duced by a similar amount of 0.9 per cent sodium chloride solution, and suggests that sucrose affects fluid reabsorption in the proximal tubule.

Any dilated surface tubule with its axis parallel to that of the pipette was selected and the point thrust into it; if the point were properly bevelled and the renal capsule not too thick the insertion could be made without tearing the tubule wall, damaging its capillaries, or penetrating into a deeper layer of tubules, though these accidents often occurred. The most perfect punctures were made in the rat by simply pressing the pipette tip against the tubule wall and allowing the small respiratory excursions to tease a hole for it. With the pipette inside the tubule lumen a short column of red oil was injected; this demonstrated the proper position of the pipette and, if it subsequently moved along the tubule into further convolutions, proved that there was a flow of fluid down the tubule. When the oil column had taken up a position distal to the site of puncture, the collection of fluid was commenced, the pressure in the collecting system being so adjusted as to immobilize the oil. The immobility of the oil column provided assurance that all of the fluid descending the tubule was being collected and that there was no contamination by fluid which had passed distal to it. In a few experiments metallic mercury has been substituted for the oil; this provides a more certain block but has been impossible to inject with consistent success.

There can be no doubt that fluid entering the pipette originated from within a nephron, for no appreciable amount of fluid can be collected from the normal kidney surface or from the kidney substance unless the pipette be thrust into a tubule lumen or damage a blood vessel. We have seen no indication of large currents of interstitial fluid (7) and there are no obvious spaces on the kidney surface unoccupied by tubules or blood vessels.

A number of factors combined to make the collections less simple than this description perhaps suggests. In the first place, the mere fact that the tubule appeared distended with fluid did not necessarily mean that there was an active flow within it, for frequently the injected oil would move onwards sluggishly or not at all; under these circumstances other tubules were punctured until one was found from which fluid could be collected. A fluid collection, once begun, continued steadily unless some extraneous factor intervened. In this observation and in the observation that pulsatile movements at the proximal end of the oil column, apparently transmitted to it from the glomerulus, continued without interruption, we find arguments against the existence of intermittent glomerular activity in our preparations. A second difficulty lay in the very high intratubular pressure which was particularly prominent during the infusion of hypertonic sucrose solutions. Under these circumstances the injected oil column, which at first had started down the tubule, would rush suddenly and violently back towards the pipette and emerge on the kidney surface. We

attributed this to a leak at the point of puncture and the consequent collapse of the punctured nephron by its distended neighbors. When saline infusions were substituted for sucrose, or all infusions omitted, the difficulty was encountered less often. A third difficulty lay in the tendency of the pipette point to become obstructed by some particle within the lumen or by contact with the tubule wall. When this occurred, the oncoming fluid either pushed the oil column on down the tubule or emerged on the kidney surface; in the latter event it often came with sufficient force to detach the capsule and form a visible drop of fluid around the point of puncture. The collection could be continued if the pipette were withdrawn from the lumen and the fluid picked up as it emerged from the tubule and lay beneath the oil which covered the kidney surface. Fourteen per cent of our collections have been made in this fashion. We deprecate the technique, for there might have been an admixture with the thin layer of surface fluid which may cover the kidney, but two points minimize the dangers introduced by this fluid. It was only present in considerable amounts when the renal capsule had been removed and in the immediate vicinity of the points upon which pressure was being exerted. It was readily distinguished from tubule fluid since it contained about 1.0 per cent protein, and reducing substances and chloride in the concentrations anticipated in an ultrafiltrate of blood plasma.

We have described the technique employed in the collection of fluid from any dilated surface tubule, chosen at random. Almost invariably these tubules proved to be proximal convolutions and in the middle third of this segment. The last third dips deeply into the kidney substance to join the loop of Henle, and is inaccessible by our technique (figs. 5–10). The first third appears occasionally on the surface and a special procedure was devised to identify it. A pipette containing air below the mercury and, at its extreme tip, a little oil, was thrust into a surface tubule. After the oil was injected and allowed to flow distal to the punctured point, a column of air was forced into the tubule. The air, prevented from going distally by the oil column, distended and outlined the coils of tubules on the glomerular side of the puncture. When the proximal end of this air column happened to be in a surface tubule this point was selected as the site of collection, with the assurance that it was on the proximal side of the original segment by the length of the air column. The technique was demanding but it provided a number of collections from the first third of the proximal tubule. It had the disadvantage that the sudden distension with air appeared to damage the tubule wall; during the subsequent experiment this portion of the tubule often looked white and some cellular detritus was found in the distal tubule at the time of its dissection. We have not felt that this damage disqualified the experiment, for the collection was made proximal to the region which had been distended. No evidence of similar damage from the

simple act of puncture or the injection of oil has been seen, other than the local tear in the tubule wall at the point of penetration. The glomerulus and the portion of the tubule proximal to the site of collection remained completely untouched and usually well below the kidney surface.

These methods for the collection of fluid from proximal tubules have proved quite satisfactory, especially in the rat. The distal tubules have presented particular difficulties, as yet only partially solved.

Collection of fluid from distal convolutions. Scattered over the surface of both guinea pig and rat kidneys there are occasional distal tubules which, coming to the surface, make but a single loop there and then descend again into the kidney substance (figs. 5–10). Their infrequency in comparison with proximal convolutions is indicated by the fact that only 3 out of 92 tubules, punctured at random, proved to be distal segments. Some special method of identification had to be designed for, though their diameter is smaller than that of proximal convolutions in a ratio of 2:3 when the measurements are made after death in dissected specimens (table 1), this difference is either absent or indistinguishable during life when the tubule lumina are dilated. We attempted to utilize the preferential vital staining of proximal convolutions by trypan blue in several series of experiments on guinea pigs and rats, hoping that the distal segments would appear as unstained tubules against a blue background. The attempts were unsuccessful for, though differentially stained, the contrast between the two segments could not be seen in the living animal.

It has, however, proved possible to identify distal tubules by the use of phenolsulfonephthalein. Six milligrams were injected intravenously and, 5 minutes later, the ureter was clamped to produce a maximum concentration of the dye. Under these circumstances the lumina of scattered tubules throughout the field became deeply colored and this color was particularly well seen when the tubule, ascending directly to the surface, allowed one to observe a column of fluid in depth. These colored tubules have uniformly proven to belong to distal convolutions. One such tubule was selected, its position relative to its surroundings noted, the clamp removed from the ureter, and puncture subsequently performed. Identification by this method involving, as it did, coloration of the tubule fluid made certain analyses impossible. The collection of satisfactory specimens has also been complicated by the small amounts of fluid available and the difficulty of establishing an adequate block. In 3 successful experiments the average volume collected has been only 0.07 c.mm. and its rate of collection 0.31 c.mm. per hour. While such small volumes were anticipated on theoretical grounds and were consistent with the demonstration of fluid reabsorption in the proximal tubule (6) they have imposed added difficulty on our analytical technique. When an oil column was injected into a distal tubule it would move onward in the direction of a collecting duct but then,

as collection of fluid was started, would return to the pipette point and often take up a position proximal to it. Under these circumstances of course the collected fluid was derived from distal to the point of puncture and the results of its analysis became meaningless. This difficulty was presumably associated with the low intratubular pressure and the extremely slow rate of flow which exists within the distal convolutions. Our methods for identification of distal tubules and for the collection of fluid from them are, then, still imperfect.

Methods of identifying the site of puncture. The collection and analysis of fluid possessed little or no significance unless the site of the collection could be accurately determined. The problem presented difficulties. In the earlier stages of the investigation attempts were made by our former colleague, Prof. Rudolf Kempton of Vassar College, to identify the punctured tubule by examining serial sections of a block of kidney tissue which contained the nephron in question. The method was very time-consuming and rarely provided more information than that the puncture was in a proximal or distal segment. Recourse was therefore had to the technique of maceration and dissection.

At the conclusion of each experiment, a pipette containing a 1:10 dilution of "soluble" india ink was reinserted into the tubule from which fluid had been collected, and a small quantity of ink injected. The kidney was then placed in 10 per cent formaldehyde. After two or three days of fixation it was removed and the small ink spot lying within the tubule and beneath the capsule around the puncture wound was located with a hand lens. A wedge shaped segment of kidney extending well into the papilla and containing the punctured nephron was then excised, care being taken to leave about 5 mm. of tissue around the central-lying ink mark. This block was placed in a stender dish containing concentrated hydrochloric acid and allowed to macerate until sufficiently softened for dissection. The length of the time required depends on the room temperature and the specimen therefore must be carefully watched, for a few hours' excessive maceration will destroy the structure of the nephrons completely. Under the conditions of our laboratory from one to three days was found to be most favorable. The acid was then poured from the softened tissue and it was rinsed by decanting with several changes of distilled water.

Dissection and isolation of the nephron was done under water beneath the binocular microscope at a magnification from 20 to 60 times, in the same dish which had contained the specimen from the beginning of the maceration. Strong direct light against a black background was found most useful during dissection, though transmitted light proved helpful in locating the ink within the tubule. Steel needles, frequently cleaned with an emery cushion to avoid the stickiness of the softened tissue, were used to untangle the marked nephron. No description of this procedure is possible, but its

progress is shown in figure 2. The most hazardous part of these manipulations was the final separation and disentanglement, without breakage, of

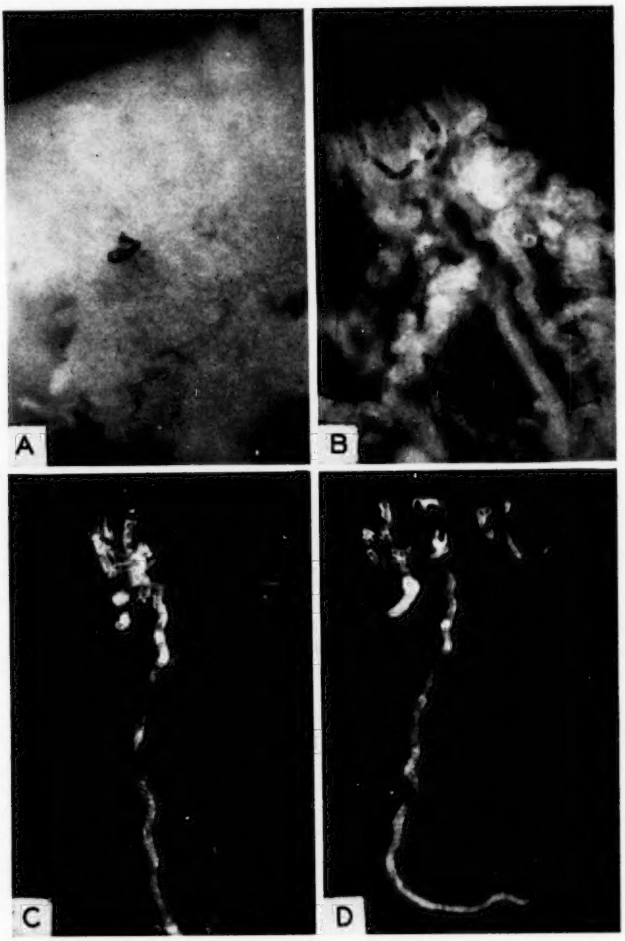


Fig. 2. Progress of dissection of a nephron (expt. 41 (6)). A, the surface of the kidney of a guinea pig after maceration showing ink in lumen of tubule. B, preliminary separation of cortical tubules; loops of proximal convolution containing ink are clearly seen. C, the isolated complete nephron showing in D, the final separation of the distal from the proximal convolution. Magnification A, C and D 22×; B, 32×.

the distal convolution from the redundant coils of the surrounding proximal convolution (fig. 2d).

Several features of the present experimental problem impose especial difficulties in the dissection of the punctured nephrons. The first and greatest of these is the disturbing fact that it is one specific nephron that must be successfully manipulated and not, as in most morphological work, a representative specimen of no peculiar value. Secondly, since quantitative measurements are to be made on the final specimen, the nephron must be isolated completely and in continuity from the glomerulus to the collecting tubule. The tenuous thin portion of Henle's loop may break at one point but no portion of it can be lost. Thirdly, the tortuous loops of

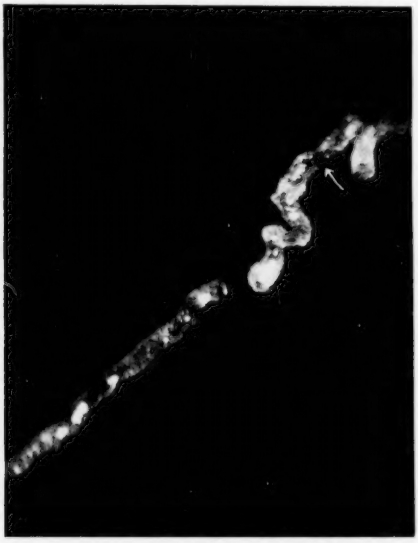


Fig. 3. The terminal portion of a proximal convolution from the kidney of a guinea pig (expt. 55 (6)). The arrow points to the small nick where the pipette entered. From this point downwards the lumen of the tubule can be distinguished and in it are oil droplets of varying size. The apparent break in the tubule is caused by an opaque rod which was laid on it for purposes of immobilization. Magnification 32×.

the convoluted tubules must be straightened out sufficiently, yet not broken, to allow an inspection of their surface throughout their entire length so that the point of entrance of the pipette with its ink mark can be examined, and the remainder of the tubule inspected for possible evidence of accidental trauma to some point other than the original insertion of the pipette. The position of the oil droplets blocking the lumen must also be determined and the adequacy of the blocking estimated by their size and number. Finally, all these manipulations were made more difficult in the experiments under consideration by the presence in the lumen of the tubule of oil or mercury. In the former case the tubules, normally of the same

specific gravity as the suspending water and therefore motionless, strove constantly to rise to the surface entangling themselves in the dissecting needles; in the latter they sank inert and heavy to the bottom of the dish and broke into fragments if roughly disturbed from this anchorage.

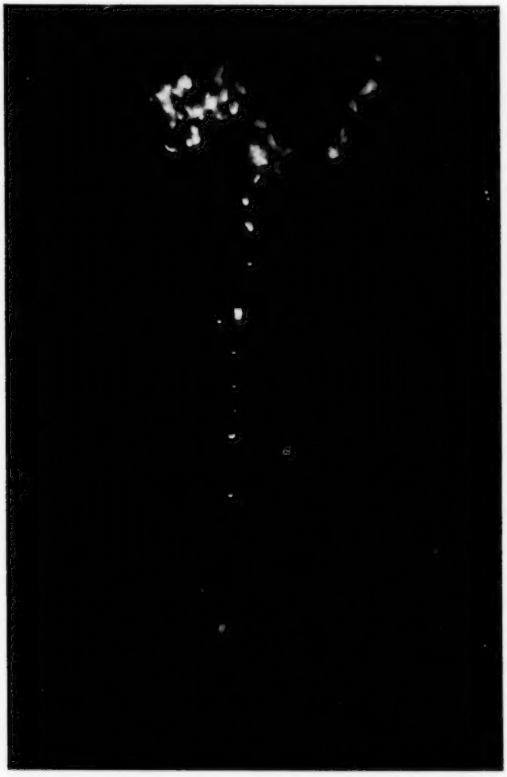
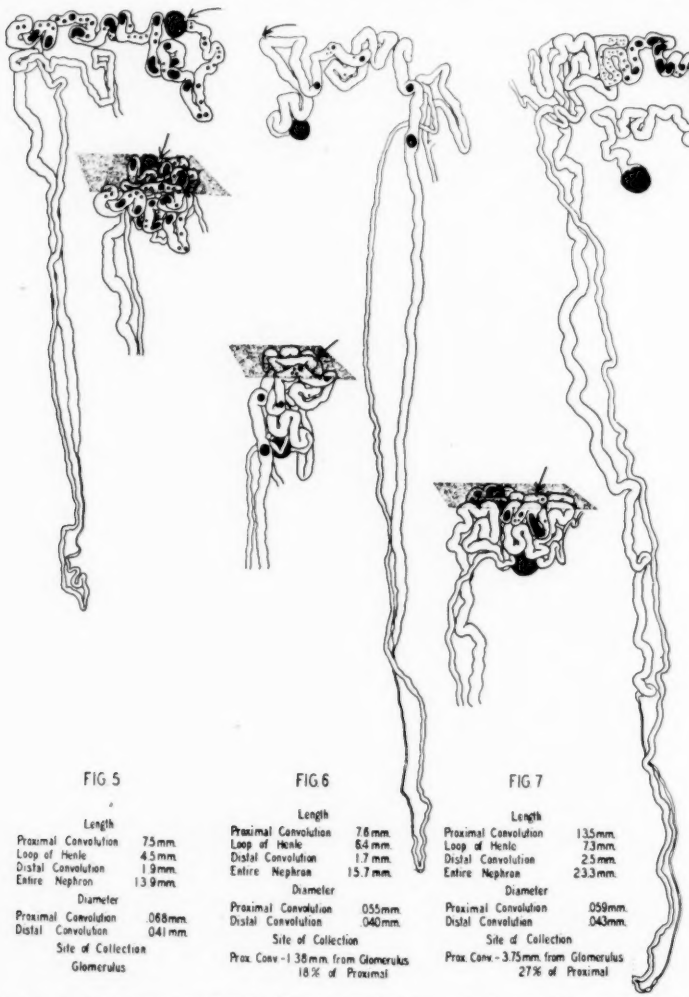


Fig. 4. The complete nephron from experiment 16 (6). Oil droplets are seen distending the tubule lumen throughout the lower four-fifths of the proximal convolution. There are no droplets in the remainder of the nephron. This relatively small nephron came from an unhypertrophied normal guinea pig kidney. Magnification 32×.

When these difficulties had been surmounted stereoscopic photographs at a magnification of 16 and 23 times were made. This gave an objective and permanent record of the morphological findings, as it is impossible to mount and preserve the delicately fragile original specimen. In most cases the photographs showed clearly the hole in the tubule at the point of entry of the pipette (fig. 3) and the occluding droplets of oil (fig. 4).

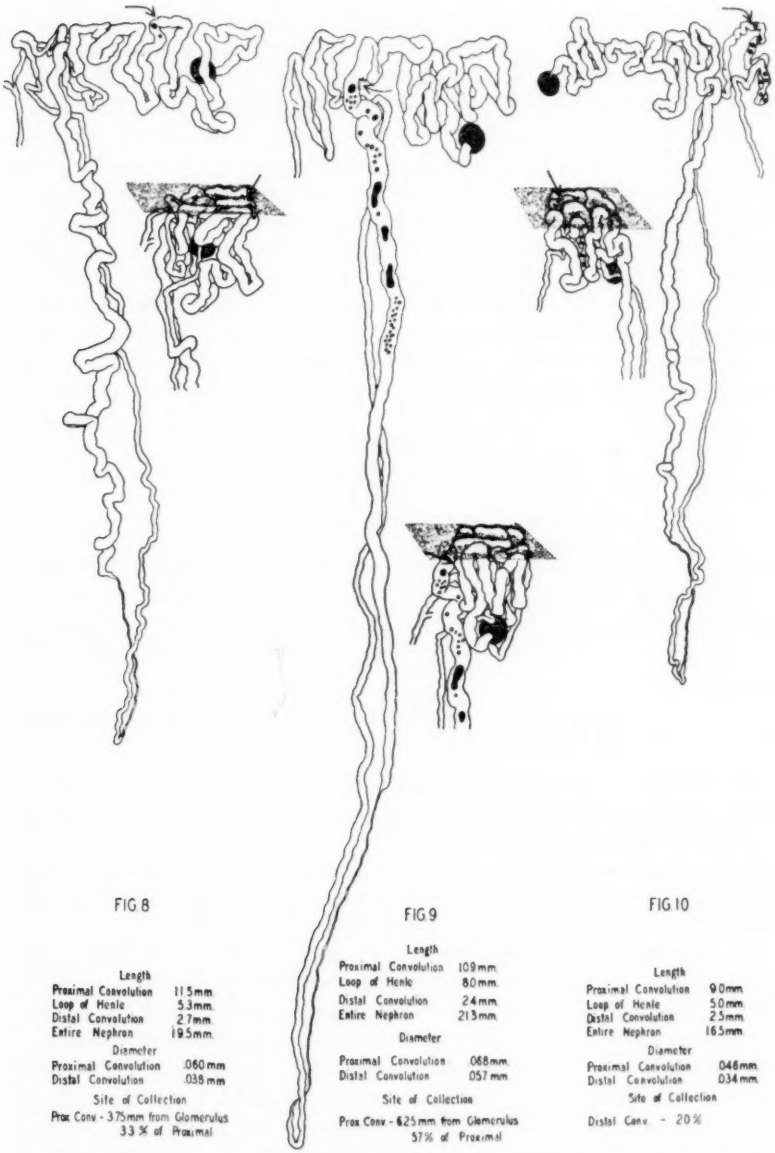
Limitations of depth of focus are noticeable however when photographing

a floating specimen, so that photographs are unsuitable for quantitative measurement. For this purpose a camera lucida outline drawing of the



Figs. 5, 6, 7. Camera lucida drawings of representative nephrons after microdissection showing the point of entrance of the pipette (arrow) and oil droplets in the tubule lumen. The small inserts show the position occupied by the loops of the convoluted tubules before microdissection. Figure 5, guinea pig; figure 6, guinea pig, experiment 13 (6); figure 7, rat, experiment 9 (6). Magnification 23×.

nephron at a magnification of 80 times was made, showing the point of pipette entrance and the site of oil and ink (figs. 5–10). In its preparation



Figs. 8, 9, 10. Camera lucida drawings of representative nephrons after microdissection showing the point of entrance of the pipette (arrow) and oil droplets in the tubule lumen. The small inserts show the position occupied by the loops of the convoluted tubules before microdissection. Figure 8, rat; figure 9, guinea pig, experiment 55 (6) (cf. fig. 3); figure 10, rat, experiment 58 (6). Magnification 23×.

a source of error in measurement, namely, the effect of foreshortening produced by the coiling of the loops of the convoluted tubules, was removed by placing thin glass capillary tubes across the floating tubule so that its loops were flattened down against the bottom of the dish in a single plane.

The actual measurement of the length of the various portions of the nephron was made on this drawing by means of a map measure with a small wheel with which the straightened and flattened twistings of the tubule could be easily followed. Measurements of the diameter of the tubule in various segments were made on the actual specimen at a magnification of 43 times with a filar micrometer. The mean of at least ten measurements was used as the final expression of the diameter, a value sufficiently accurate for the purpose of these experiments, though it must be recognized that such an evaluation ignores the fact that in the hypertrophied proximal convolution the straight terminal portion, comprising

TABLE 1

Measurements of hypertrophied nephrons

	PROXIMAL CONVOLUTION		LOOP OF HENLE	DISTAL CONVOLUTION	
	Length	Diameter	Length	Length	Diameter
	mm.	*mm.*	*mm.*	*mm.*	*mm.*
Rats (37)	11.4 (6.8–19.3)	0.058 (.04 –.080)	6.7 (5.0–9.8)	2.4 (1.2–3.0)	0.040 (.032–.050)
Pigs (37)	8.0 (5.5–11.6)	0.056 (.039–.079)	5.7 (3.8–8.5)	1.7 (0.9–2.8)	0.044 (.034–.062)

about one-third of its length, may be twenty-five per cent thicker than the mean diameter of the convolution as a whole. A summary of these measurements is shown in table 1.

Besides the measurement of the length of the various portions of the nephron, the distance of the point of entrance of the pipette from a glomerulus was also recorded by means of the map measure.

This completed the morphological data on the nephron. The final procedure, accomplished in part during the course of the earlier dissection, was the isolation and inspection of neighboring nephrons to make certain that they had not been entered by the pipette or torn so that a source of contaminating tubule fluid had been produced. Ink granules which had leaked from the punctured tubule lumen were found not uncommonly in the interstitial tissue and on neighboring tubules, but its presence on the surface of an intact tubule rather than in its wall or its lumen allowed an easy decision as to whether a possible contamination could have occurred. In five instances such accidents were found to have happened; tears were

seen in contiguous tubules or their lumens contained either ink or, more frequently, oil droplets which showed plainly that more than one nephron had been involved in the experimental puncture.

To sum up the advantages of the morphological procedure it may therefore be stated that an objective and permanent record which allowed accurate quantitative measurement was obtained; that the position of the point of entrance of the pipette into the tubule lumen relative to the remainder of the nephron could be accurately determined; that the adequacy of the oil block could be demonstrated and that errors due to entrance into more than one nephron, errors not appreciated during the course of the experiment, could be positively eliminated.

SUMMARY

Methods have been developed for observing the kidney surface in anesthetized mammals and for collecting fluid from single glomeruli, proximal tubules, and distal tubules. Sufficient amounts of fluid can be collected for quantitative analysis by ultramicro methods and the precise *locus* of the collection can be determined.

It is unnecessary, but a pleasure to us, to remark that these experiments are a direct and logical continuation of those initiated by Prof. A. N. Richards many years ago and to record his continuous encouragement and advice in their execution.

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THE COLLECTION AND ANALYSIS OF FLUID FROM SINGLE NEPHRONS OF THE MAMMALIAN KIDNEY[1]

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Methods have been developed for the collection of fluid from single glomeruli and tubules of the mammalian kidney (1). The amounts of fluid which can thus be collected, though small (from 0.03 to 0.70 c.mm.), were sufficient for quantitative analysis by ultramicro methods (2), and such analyses have been performed in a series of 92 experiments upon rats, guinea pigs and opossums. It was the design of the experiments to learn some of the changes which occur as fluid descends a tubule from the glomerulus toward the ureter and to establish the site of these changes, the same type of experimentation which has yielded considerable information in the amphibian kidney (3). In the present instance it has confirmed existing ideas of glomerular function, established the site of glucose reabsorption, and shed some light on the extent and type of fluid reabsorption which occurs in the proximal tubule.

Methods of analysis. The analytical methods, excepting that for sodium, were devised in connection with the experiments upon amphibia and are fully described in the publications to which reference will be made. They were carried out in small glass capillary tubes. Changes in the original techniques and details pertinent to the present investigation are summarized in the following paragraphs.

Protein (2). A polished black surface was used as the background, and a micro lamp with 300 watt bulb and spherical condenser as the source of illumination, in examining for the presence of precipitate. The concentrations of protein ascribed to glomerular and tubule fluid in the text are based on control experiments in which various dilutions of guinea pig blood plasma, assumed to contain 7 per cent protein, were used. Concentrations less than 0.03 per cent could not be distinguished with certainty. The results are only quantitative in the sense that, for example, 0.05

[1] The expenses of this work have been defrayed in large part from a grant by the Commonwealth Fund of New York. The results of the experiments were reported before the American Physiological Society at Chicago in April 1941 (This Journal **133:** 480, 1941).

per cent could be readily distinguished from 0.10 per cent. The method of Shevky and Stafford (4) was used for the macro analyses of protein in bladder urine.

Reducing substances[2] (5). The accuracy of the ultramicro colorimetric method was re-examined by comparing its results with those obtained by the Shaffer-Somogyi macro method (6) in the analysis of 6 specimens of guinea pigs' and rats' blood plasma. The results of the two methods did not differ by more than 8 mgm. per 100 cc. and the average difference was 4 mgm. per 100 cc. Fluids containing more than 125 mgm. per 100 cc. were diluted previous to analysis, since full color development in capillary tubes does not occur above this concentration and since the color becomes difficult to read accurately. Analyses of volumes of tubule fluid less than 0.10 c.mm. and of blood plasma less than 0.15 c.mm. gave incomplete color development and the results of experiments in which such volumes were used have been omitted. Heparin should not be used as an anticoagulant.

In the experiments upon guinea pigs, the concentration in tubule fluid was compared with that of two specimens of blood plasma collected at the beginning and end of the experiment, their average being obtained by interpolation to the mid-point of the experiment. Because the animals were eviscerated and a considerable interval of time separated the two plasma collections, the glucose concentration of specimen 2 averaged 40 mgm. per 100 cc. less than specimen 1. This fall in glucose concentration diminished the accuracy of the average plasma value recorded in table 1. The fall was so marked following the injection of phlorhizin, that the eviscerated guinea pig could not be used for this type of experiment unless the hepatic circulation were left intact, a procedure too difficult for routine use and employed but once (expt. 31).

In the experiments upon rats, since evisceration was unnecessary, the blood glucose was quite constant, and comparison could be made with a single specimen of plasma obtained from the animal's tail at the mid-point of the collection.

The bladder urine formed by guinea pigs during the experiments had an average concentration of reducing substances of 0.06 per cent when analyzed by the ultramicro method, and 0.13 per cent by the Shaffer-Somogyi macro method. The greater part of these substances was not glucose for 6 of the same specimens, when analyzed for fermentable sugars, contained an average of only 0.019 per cent. The values for urine have been omitted from table 1 because of the presence of these non-fermentable reducing substances but they do not, apparently, appear within the proximal tubule in sufficient concentrations to affect analyses of fluids from this site.

Creatinine (7). The procedure referred to as "method B" was used both for plasma and tubule fluid. In the preparation of protein-free filtrates of plasma we employed 1 volume of plasma, 0.5 of 10 per cent sodium tungstate, 8.5 of N/25 sulphuric acid. The accuracy of the method proved to be as previously described. The comparison between tubule fluid and blood plasma in guinea pigs was made less accurate by the rapid fall in plasma concentration (averaging 0.015 per cent) which occurred between collection of the two blood specimens; the plasma figure in table 1 was obtained by interpolation to the mid-point of the experiment. In rats the plasma concentration only changed an average of 0.003 per cent during the course of an experiment, and comparison was usually made with a single specimen obtained at the mid-point of the experiment.

Osmotic pressure (8). In previous papers the results of this measurement have been designated—somewhat inaccurately— as "total molecular concentration." The measurement consists in comparison of the vapor pressures of tubule fluid and plasma

[2] The macro analyses for reducing substances, chloride and protein were made by Miss Ethol Shiels, Department of Pharmacology, University of Pennsylvania.

by Barger's capillary method for determining molecular weight. The results given in table 1 show the number of micrometer scale divisions, each equivalent to 4.7μ, by which a column of tubule fluid changed in length during 48 hours' equilibration in a glass capillary tube against heparinized blood plasma from the same animal. In each experiment a similar comparison was made between bladder urine and plasma, and a third (control) capillary was prepared in which all of the columns were bladder urine. The results of this latter comparison, omitted from the table, show an average change in length of only 1.8 scale divisions—an insignificant difference.

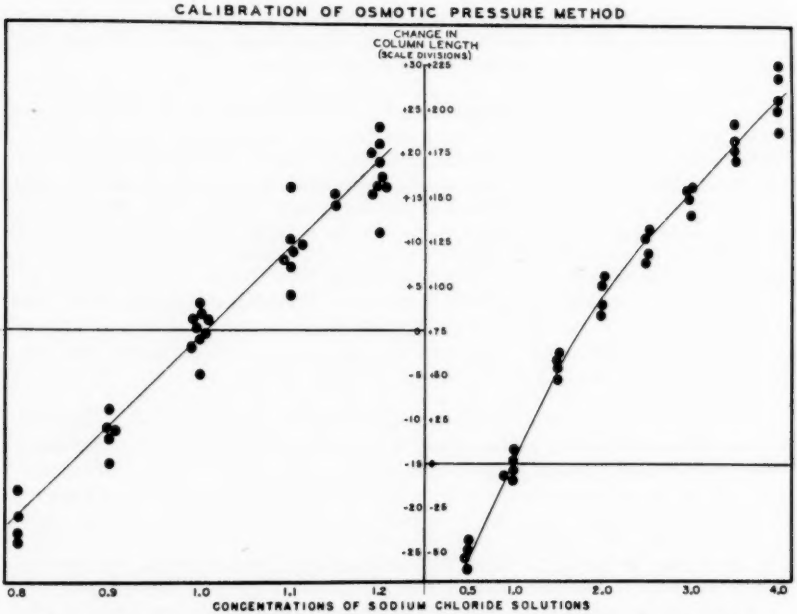

Fig. 1. Each dot represents an experiment in which blood plasma of a rat or guinea pig was compared with sodium chloride solutions of known concentrations. The ordinates represent the number of micrometer scale divisions (each 4.7μ) by which the 2 mm. column of plasma increased (+) or decreased (−) in length during 48 hour equilibration in a glass capillary tube.

In figure 3 the same results are plotted in terms of percentage differences between the fluids and plasma; this quantitative expression was obtained from a series of 11 control experiments in which columns of blood serum or heparinized blood plasma, collected from the vena cava, renal vein, or tail of normal or operated guinea pigs and rats, were equilibrated with sodium chloride solutions of known concentrations by precisely the same procedure. The results show that 10 per cent differences are readily recognizable and that the osmotic pressure of plasma is approximately that of a 1.0 per cent NaCl solution. They yield the curves shown in figure 1, reference to which enables the experimental results to be expressed as percentage differences, and they are so charted in figure 3.

In the experiments it was often necessary to use less than 0.2 c.mm. of tubule fluid; when this was the case similarly small amounts of bladder urine were used. When the difference in osmotic pressure between the two fluids being compared is less than 20 per cent, the resulting change in column length was found to be independent of the volume used; when the difference exceeds 20 per cent, the use of small volumes resulted in an osmotic pressure reading below the actual value. The great majority of the tubule fluid determinations are therefore not open to objection on this score but the osmotic pressure of the bladder urine specimens has been underestimated.

Chloride (9). The accuracy of the ultramicro method was re-examined in a series of 10 experiments on sodium chloride solutions, blood plasma and urine specimens. Each specimen was analyzed by both ultramicro and Eisenman (10) macro methods; the average difference between results by the two methods was 2.7 per cent, the maximum 4.9 per cent. The concentration in tubule fluid was usually compared with that of a single specimen of oxalated blood plasma obtained in mid-collection or at the end of the experiment. Such comparisons are justifiable for, in 2 experiments, blood specimens collected at one hour intervals showed a maximum difference of 1.5 per cent. Results are recorded in terms of milligrams of sodium chloride per 100 cc. and have been corrected for dissolved chromate and creatinine, the latter correction being only necessary in some experiments and then only in the case of bladder urine. The values reported for tubule fluid proved to be unaffected by preliminary precipitation with zinc hydroxide. Blood plasma collected from the renal vein had the same concentration as that collected elsewhere.

Sodium. An ultramicro colorimetric modification of the Butler-Tuthill (11) gravimetric method has been developed by Doctor Bott and will be described in a separate publication. Its accuracy is similar to that of the other methods employed in this investigation.

RESULTS. The results of analyses upon 59 of the 92 specimens which have been collected appear in table 1 and figures 2 and 3. Thirty-three experiments have been excluded on the following grounds: site of collection could not be identified (eleven) or could only be identified as "a proximal convolution" (seven); no precautions ("block") against contamination of the collected fluid by fluid originating distal to the site of collection (six); more than one tubule punctured (five); inadequate fluid for accurate analysis (three); one additional experiment (B-46) will be mentioned in the text. The remaining 59 experiments have been carefully reviewed and are regarded as free from known technical fault; in each the block was adequate, the site of collection accurately determined,[3] and the analysis acceptable. In the entire series of experiments, the average duration of the collection was 21 minutes and the average amount of fluid available for one or more analyses 0.27 c.mm.

We have adhered to the practice, employed in the amphibian experiments, of defining the site of collection in terms of fractions of total tubule length rather than in terms of millimeters from the glomerulus. The experiments in table 1 have been arranged in that order and the symbols in

[3] Two experiments, 7 and 12, have been included though two units were found punctured at the time of dissection since it did not appear to us that the result of either experiment could have been influenced by this accident.

TABLE 1

Results of analyses of glomerular and tubule fluid

EXPERIMENT NUMBER	MAL	COLLECTION			GLUCOSE (NORMAL)			GLUCOSE (PHLORHIZIN)				CREATININE				CHLORIDE				OSMOTIC PRESSURE	
		Site*	Am't.	Rate	Fl.	Pl.	Fl/Pl.	Fl.	Pl.	Fl/Pl.	BU/Pl.	Fl.	Pl.	Fl/Pl.	BU/Pl.	Fl.	Pl.	Fl/Pl.	BU/Pl.	Fl. vs Pl.	BU vs Pl.
			c.mm.	*c.mm./hr.*	*mgm./100 cc.*			*mgm./100 cc.*				*mgm./100 cc.*				*mgm. NaCl/100 cc.*				*scale divisions†*	
1	G. pig	Glom.	0.40	0.50	96	104	0.92													−1	+137
2	G. pig	Glom.	0.45	0.71	88	92	0.96													−2	+83
3	G. pig	Glom.	0.18	0.64	175	177	0.99														
4	G. pig	Glom.	0.19	0.38	98	102	0.96														
5	G. pig	Glom.	0.06	—								65	55	1.18	87.3						
6	G. pig	Glom.	0.24	0.66								34	33	1.03	125.7						
7	G. pig	Glom.	0.15	1.12								29	30	0.97	151.6						
		Proximal																			
8	G. pig	0.3/ 7.1	0.37	1.10	115	140	0.82													−4	+108
9	Rat	0.3/ 6.8	0.44	0.98								22	20	1.10	167.8					−6	+238
10	G. pig	1.0/ 9.8	0.06	0.40												810	647	1.27	0.51		
11	Opossum	0.7/—	0.27	1.35	65	75	0.87													+4	+108
12	Rat	1.5/12.9	0.66	3.30	170	193	0.88									700	652	1.07	0.23		
13	G. pig	1.4/ 7.6	0.26	0.98								33	28	1.18	30.6						
14	Rat	2.1/10.8	0.23	0.92	65	86	0.76									837	647	1.30	0.21		
15	Rat	2.5/11.8	0.08	0.60																−1	+193
16	G. pig	1.5/ 6.8	0.21	—	47	121	0.39														
17	G. pig	1.6/ 7.4	0.35	—	68	213	0.32													−15	+41
18	Rat	3.1/12.9	0.24	1.03				190	123	1.54	50.0					910	626	1.45	0.26		
19	Rat	3.8/13.5	0.11	—				242	179	1.35	20.4										
20	G. pig	3.1/11.2	0.30	0.67	31	92	0.34													+1	+18
21	G. pig	2.5/ 9.1	0.51	0.87	110	114	0.97													−3	+170
22	Rat	4.3/14.3	0.08	0.32																−2	+76
23	Rat	4.0/13.5	0.15	0.47								55	40	1.38	86.5	770	637	1.21	0.20		
24	Rat	3.3/10.8	0.63	1.57	80	198	0.40					49	25	1.96	—	940	—	—	—		
25	Rat	2.9/ 9.3	0.21	1.05												870	647	1.34	0.67		
26	Rat	6.5/19.3	0.58	1.51	48	171	0.28									986	677	1.46	1.08		
27	G. pig	1.9/ 6.0	0.34	1.02								35	28	1.25	37.9						
28	G. pig	2.9/ 9.1	0.13	0.52	52	93	0.56														

28	G. pig	2.9/ 9.1	0.13	0.52	52	93	0.56													
29	Rat	3.9/10.8	0.10	—											916	636	1.44	0.68	−6	−8
30	G. pig	3.1/ 8.3	0.51	1.53	38	59	0.64													+115
31	G. pig	3.1/ 8.3	0.12	0.36				390	256	1.52	8.1									
32	Rat	6.8/17.5	0.19	0.88				321	193	1.66	—	51	29	1.76	47.4					
33	G. pig	2.1/ 5.5	0.40	0.50	68	152	0.45												0	+58
34	G. pig	3.4/ 8.5	0.25	—	45	97	0.47					51	24	2.12	50.8				−7	+137
35	Rat	3.6/ 9.0	0.48	1.60																
36	Rat	5.5/13.1	0.70	1.68				144	67	2.15	63.5	54	26	2.08	58.3	940	647	1.45	0.19	−2	+161
37	Rat	4.8/11.0	0.20	0.75	25	144	0.17													
38	Rat	3.8/ 8.8	0.20	0.80				164	66	2.48	—	84	39	2.16	40.6					
39	Rat	2.6/ 6.0	0.42	1.01								77	36	2.14	38.2				−50	+49
40	Rat	5.5/12.9	0.07	—				270	144	1.88	52.6									
41	G. pig	2.9/ 6.8	0.08	0.22								63	44	1.46	23.8					
42	Rat	3.9/ 8.5	0.14	0.70								63	34	1.85	125.0					
43	Rat	4.7/10.5	0.31	0.62				440	147	3.00	40.0					805	597	1.35	0.15	−6	+139
44	Rat	3.5/ 7.5	0.06	1.20								34	21	1.64	—					
45	Rat	5.3/11.0	0.06	—				282	113	2.74	29.3									
46	G. pig	4.3/ 9.0	0.17	0.54												930	644	1.45	1.04	
47	Rat	4.4/ 8.8	0.09	0.26												866	596	1.45	0.17	
48	G. pig	4.0/ 8.0	0.17	0.34								82	44	1.87	134.6					
49	Rat	5.8/11.5	0.22	0.78				234	91	2.53	39.4								−6	+112
50	Rat	5.5/10.8	0.10	0.60				220	86	2.56	54.4									
51	Rat	5.5/10.5	0.05	1.00															+2	+221
52	G. pig	2.9/ 5.5	0.32	0.64								53	36	1.47	41.0					
53	G. pig	6.4/11.2	0.41	1.12	32	126	0.25													+178
54	Rat	8.0/14.0	0.45	0.60				280	118	2.38	59.6					900	652	1.38	0.22	−3	+65
55	G. pig	6.3/10.9	0.31	1.10	18	118	0.15													−2	+145
56	G. pig	7.7/10.0	0.24	0.39	22	91	0.24													−3	+111
		Distal																		
																			−24	+144
57	Rat	0.5/ 3.5	0.05	0.48															−7	+104
58	Rat	0.5/ 2.5	0.03	0.06															−25	+65
59	Rat	0.5/ 2.0	0.12	0.40																

Abbreviations: "G. pig" = guinea pig; "Glom." = glomerulus; "Fl." = glomerular or tubule fluid; "Pl." = blood plasma; "BU" = bladder urine. In columns describing glucose analyses "Phlorhizin" means that the drug has been administered to these animals before the experiment, "normal" that it has not been administered.

Sodium analyses: Tubule fluid and blood serum were analyzed for sodium in experiments 12 and 26; expressed as mgm. Na per 100 cc., the concentrations in tubule fluid were 350 and 340, in serum 334 and 334; the fluid/serum concentration ratios were therefore 1.06 and 1.02. In these serum analyses, as in those for glucose, creatinine and chloride in plasma, a correction of 7 per cent has been added so that the results may appear in terms of plasma water.

* The numerator of the fractions in this column is the measured distance, in millimeters, from the beginning of the convolution to the point of the fluid collection; the denominator is the measured total length of the convolution.

† The recorded changes are those of the central fluid column, concomitant changes in peripheral plasma columns being omitted.

figures 2 and 3 have been plotted on that basis. The figures in column 3 of the table will permit rearrangement of the data on the basis of absolute length.

Since a comparison is being made between a protein-free fluid and blood plasma, the concentrations in the latter should be reported on the basis of plasma water. A plus correction of 7 per cent has therefore been added to the results of the plasma analyses. No such correction is indicated in the case of osmotic pressure measurements (12).

Protein. Forty-one specimens of fluid from glomeruli and proximal tubules were tested for protein. No protein was found in 25 specimens; the circumstances of the test were such that 8 of these would have been positive had they contained 0.03 per cent, 17 had they contained 0.08 per cent. Of this group two were collected from glomeruli and two from within 1 mm. of a glomerulus. Sixteen specimens gave positive tests for protein; 14 of these contained less than 0.2 per cent and 9 less than 0.08 per cent. Twelve specimens of bladder urine, collected at the end of the experiments, contained an average of 0.17 per cent.

These results confirm the present belief that normal glomerular fluid contains either no protein or, at most, very small amounts. They do not distinguish between these two alternatives, but they have the virtue of excluding any third possibility. It was demonstrated that slight mechanical trauma to glomerular capillaries, far short of actual rupture, will make them permeable to gross amounts of protein.

Reducing substances. The 22 experiments summarized in table 1 and figure 2 prove that glucose occurs in glomerular fluid in concentrations similar to those in blood plasma and is reabsorbed as fluid flows through the proximal tubule. The site of glucose reabsorption is thus identical in these animals and in amphibia (16) and, as in amphibia, the process is practically completed by the first half of the proximal convolution.

The amount of glucose reabsorbed by the kidney tubules is not fixed but, like other aspects of renal function, is capable of variation. It increases, for example, during moderate rises in plasma glucose concentration as the absence of glycosuria under these circumstances indicates. Two mechanisms might be concerned in such an increase of the reabsorptive process. As plasma concentration rises, fluid reaching the latter part of the proximal convolution, normally free of glucose, might now contain it and this portion of the convolution share in the reabsorption. Alternatively, larger amounts of glucose might be reabsorbed by the earlier part of the proximal segment. In the present experiments this second alternative would seem to be demonstrable. When collections from comparable levels of the tubule are examined (table 1, expts. 16 and 17, 28 and 33) the tubule fluid/plasma ratios are seen to be very similar though the plasma glucose concentrations differ widely. Unless large and fortuitous changes in glomerular filtrate volume occurred in these nephrons, the results prove that the earlier parts

of these tubules reabsorbed more glucose when it was presented to them in increased amounts.

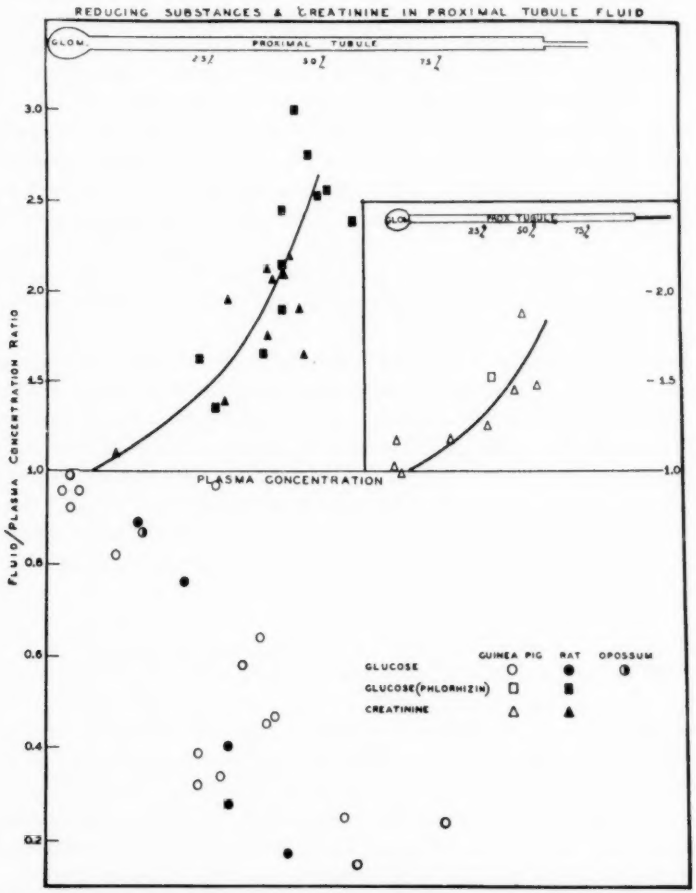

Fig. 2. Chart showing concentration ratios between blood plasma and fluid collected from glomeruli and proximal tubules with respect to exogenous creatinine, and to reducing substances with and without the preliminary injection of phlorhizin. Sites of fluid collections can be identified by reference to the diagrammatic tubules. The inset contains results of experiments upon guinea pigs, separated from those upon rats because the fluid/plasma concentration ratios were of a different order. The curves are drawn to indicate the concentration ratios which would result from the reabsorption of 12.5 per cent (inset 7.5 per cent) of the fluid of glomerular filtrate by each 10 per cent of the tubule length.

In the single experiment (table 1, expt. 24) in which creatinine analyses permitted calculation of glomerular filtrate volume in the nephron

under examination, it was computed that glucose was reabsorbed at the rate of 0.001 mgm/mm. of proximal tubule/hour.

Fluid reabsorption. In 11 experiments on rats, and 1 on a guinea pig, 200 mgm. of phlorhizin per kilogram were injected subcutaneously 30 minutes before the preparation was begun. The fluids, subsequently collected from portions of the proximal tubules, were analyzed for reducing substances and the results of these analyses appear in table 1 and figure 2. As has been believed, and demonstrated in amphibia (16), the reabsorption of glucose by the proximal tubule is arrested. If this alone occurred the concentration in tubule fluid would approximate that in blood plasma but, since it rises markedly above plasma concentration, some additional process must be involved. Only two processes could be concerned; the first, an active secretion of glucose by the proximal tubule, does not merit consideration (16); the second is the reabsorption of fluid and this we believe the experiments to demonstrate.

The problem of fluid reabsorption has also been examined by determinations of exogenous creatinine. In 18 experiments on rats and guinea pigs, following the subcutaneous injection of 300 to 500 mgm. of creatinine/kgm., fluid was collected from glomeruli and portions of the proximal tubules. The results of these analyses, and their comparison with plasma also appear in table 1 and figure 2. As was the case with glucose, the concentrations in tubule fluid rise markedly above those in plasma. In 4 experiments, both analyses were performed on the same specimens of tubule fluid with the following results:

	TUBULE FLUID/PLASMA CONCENTRATION RATIOS			
	B-42	32	36	38
Creatinine	1.55	1.76	2.15	2.16
Glucose	1.42	1.66	2.08	2.48

The similarity between the results obtained by these two methods make it unnecessary to consider the possibility of creatinine secretion.[4] The fluid/plasma ratios with this substance, as with glucose, may be attributed to the reabsorption of fluid.

[4] It could be argued that some creatinine was secreted by the normal rat but that secretion was abolished by phlorhizin in these 4 experiments. Such an effect of phlorizin has been described in man (13) but it has not been reported in other mammals and an examination of table 1 does not suggest that phlorhizin has lowered the concentration ratio of creatinine in these experiments (expts. 35 and 36, 38 and 39). Simultaneous inulin and creatinine clearances have not been performed in rats but the similarity between these two measurements in other lower mammals (14) (15) is also acceptable evidence against the secretion of creatinine.

The two series of experiments[5] may then be considered together. They demonstrate the reabsorption of very large amounts of fluid by the proximal tubules of both animals. It will be observed that the concentration ratios rise more and more abruptly as fluid is collected further and further from the glomerulus. This suggests that similar amounts of fluid are reabsorbed per unit of tubule length, for such a reabsorption would produce a progressively greater effect upon the concentration ratios as these ratios increase. The suggestion was tested by drawing the curves in figure 2 (see legend) and, since the data fit them, the suggestion may be accepted. In rats about 12.5 per cent, in guinea pigs about 7.5 per cent of the fluid of glomerular filtrate is reabsorbed by each 10 per cent of the first half of the proximal convolution.

The average concentration ratios, at a point half way down the proximal tubule, demonstrate that 60 out of each 100 cc. of glomerular fluid has been reabsorbed in rats, 40 out of each 100 in guinea pigs. It is technically impossible (1) to collect fluid from the end of the proximal convolution but it seems proper to assume that the fluid reabsorption continues, though necessarily to a lesser extent in rats, throughout the second half of this segment. The reabsorption of a total of 80 out of each 100 cc. of glomerular fluid, with a consequent concentration ratio of 4.0, would seem a conservative estimate. If this estimate be allowed, it follows that only 18 or 19 cc. of fluid need be reabsorbed by the entire loop of Henle and distal convolution to account for the concentration ratios observed in the bladder urine of these animals. This predominance of the proximal tubule in fluid reabsorption was not anticipated. It is the reverse of the situation which has been demonstrated in amphibia (16). It supports the prescient suggestion of Homer Smith (17) that "a great part of the water might be reabsorbed in the proximal tubule."

It is pertinent to inquire the effect of changes in the volume of glomerular filtrate upon this fluid reabsorption, for the question constantly arises in studying the rôle of the glomerulus in diuresis. When glomerular filtrate increases, does the tubule continue to reabsorb the same amount of fluid as previously or does it reabsorb the same percentage of the fluid now

[5] Two experiments are selected for special mention. The effect of phlorhizin in abolishing glucose reabsorption was shown particularly well in experiment B-42, omitted from table 1 because the site of collection was not accurately determined. Two collections were made from the same site in the same proximal tubule; the first, previous to the intravenous injection of phlorhizin, showed a fluid /plasma ratio of 0.48; the second, after the injection, a ratio of 1.42. Experiments 40 and 18 illustrate well the increasing concentration ratios of fluid collected at increasing distances from the glomerulus; two collections were here made from the same proximal tubule; in the first, 43 per cent of the distance from glomerulus to loop of Henle, the ratio was 1.88; in the second, 24 per cent of the same distance, it was 1.54. A drawing of this nephron appears in figure 4.

reaching it in greater amounts? In the present experiments the percentage rather than the amount of reabsorption remained constant; fluids descending the tubule at very different rates ("rates of collection") showed very similar degrees of concentration (table 1, expts. 35 and 38). This observation applies only to the site of experimentation: early proximal tubule. It does not imply that the same percentage reabsorption would persist in the presence of higher rates of glomerular filtration, but it serves as another example of the adaptability of tubule activity which was noted in connection with glucose reabsorption.

The validity of using these concentration ratios in tubule fluid to measure fluid reabsorption may be examined by inquiring what demands they make upon the rate of glomerular fluid formation. To supply the amounts of creatinine and glucose found in tubule fluid, glomeruli in guinea pigs must have formed an average of 1.00 c.mm. of fluid/hr. (7 expts.) with a maximum of 1.37, and in rats 1.96 c.mm./hr. (17 expts.) with a maximum of 3.76.[6] These demands are reasonable, for fluid has actually been collected from a guinea pig glomerulus at the rate of 1.1 c.mm./hr. and from very close to a rat glomerulus at 3.3 c.mm./hr.

It may seem that a more direct way of measuring fluid reabsorption than the use of concentration ratios would have been to compare the amounts of fluid collected from various levels of the proximal tubule. The technical difficulties involved in making these measurements, and the improbability of the assumption that glomeruli in different animals are forming fluid at similar rates, diminish the significance of this procedure. Nevertheless a general trend in the anticipated direction appears when the experiments are grouped according to the sites of collection; this trend is sufficiently definite in the experiments on rats to confirm the proof that considerable fluid is reabsorbed in the proximal convolution:

	AVERAGE RATE OF COLLECTION (CU. MM./HR.) FROM PROXIMAL CONVOLUTION		
	First fifth	Second fifth	Third fifth
Rats	1.7 (3)	1.1 (8)	0.8 (13)

Additional information as to the character of this fluid reabsorption is obtained by an examination of the osmotic pressure estimations.

[6] When the amounts of fluid formed per glomerulus per hour are calculated from the bladder urine analyses in these experiments, the average, in 17 guinea pigs, was 1.3 and in 17 rats, 2.1 c.mm. The data are imperfect for the procedure was not designed for the performance of clearance experiments, but they may be regarded as supporting evidence for the above results. Incidentally, since they are computed on the basis of all glomeruli being in constant activity, their agreement with the results from single nephrons constitutes evidence against intermittence. (See also (1, p. 569).)

Osmotic pressure. The osmotic pressure of fluid from glomeruli and the proximal tubules of rats, guinea pigs, and an opossum was directly compared with that of blood plasma in 21 experiments. The results, recorded in table 1 and figure 3, show substantial identity between the two fluids. Eight additional experiments gave similar results but are omitted because the sites of collection were not accurately determined. These results are analogous to those which have been described in the case of amphibia (8). Since the tubule contents, during and after the reabsorption of large

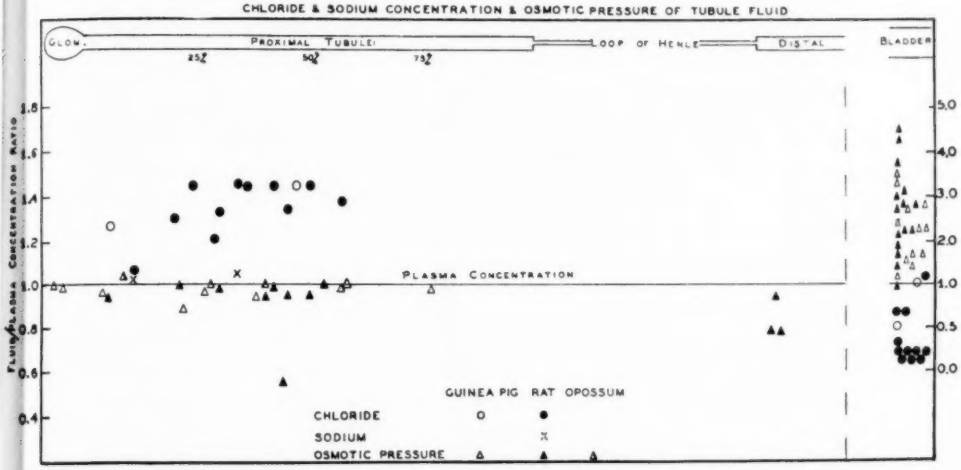

Fig. 3. Chart showing concentration ratios between blood plasma and fluid obtained from glomeruli, proximal and distal tubules and bladder with respect to osmotic pressure and concentrations of chloride and sodium. The ordinates on the left apply to glomerular and tubule fluids, those on the right to bladder urines. The values for osmotic pressure were obtained by interpolating the observed change in column length on the curves of figure 1. Site of fluid collections can be identified by reference to the diagrammatic tubule.

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Three specimens were collected from the *distal* convolutions of rats under

[7] The statement must be qualified to this extent; the fluid is certainly not hypertonic to blood plasma, but a number of the experiments suggests that it is slightly hypotonic. In all but 2 experiments the difference is scarcely beyond the error of the method but, if it be accepted as valid, indicates that these fluids have an osmotic pressure about 5 per cent less than that of plasma. In 2 experiments (17 and 39) the difference is far outside any error in the method and both tubule fluids are definitely hypotonic to blood plasma. No technical errors were recognized. It may be significant that the bladder urines in these 2 experiments have lower osmotic pressures than the great majority of such specimens.

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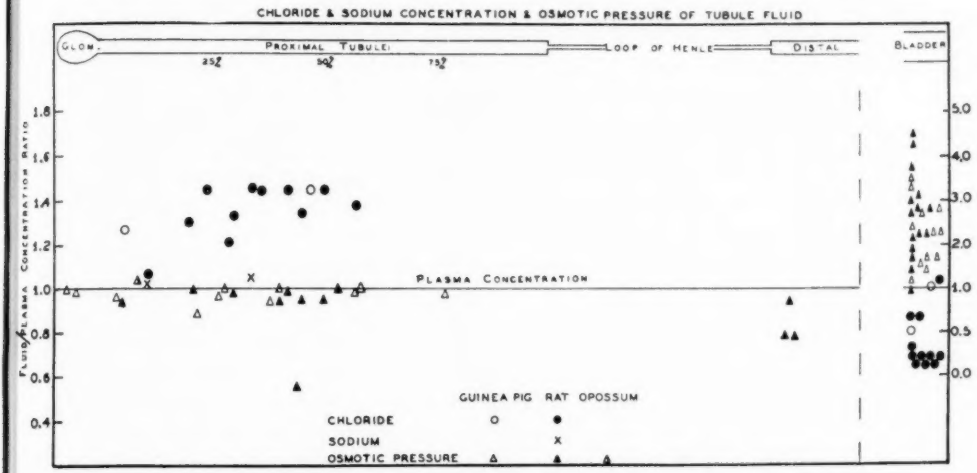

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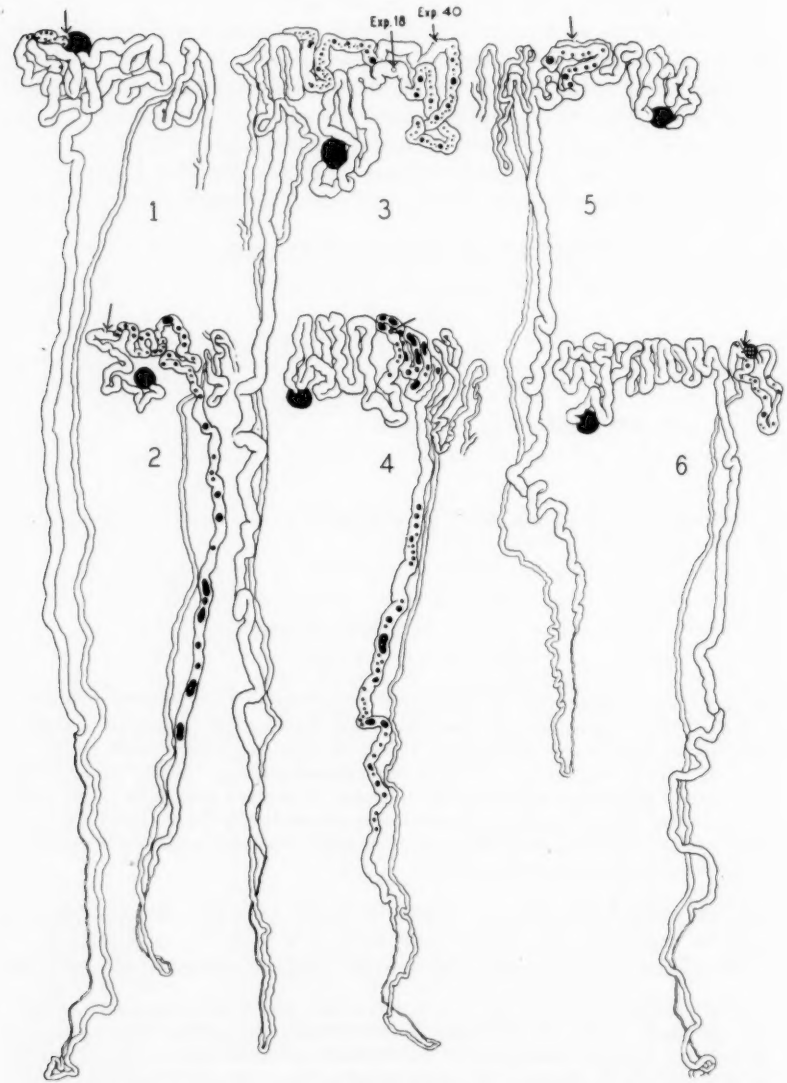

Fig. 4. Camera lucida drawings of nephrons after microdissection showing the point of entrance of the pipette and the oil and mercury blocks in the tubule lumen beyond the site of collection. The arrow shows point of entrance, solid black drops

circumstances which prevented contamination by fluid from the collecting ducts (table 1, expts. 57 to 59). Two of these specimens (and one additional specimen which was collected without this precaution) had osmotic pressures definitely below those of blood plasma, being approximately equivalent to an 0.8 per cent sodium chloride solution (fig. 1). In both cases the osmotic pressure of bladder urine was well above that of blood plasma. More data are required before any conclusions may be drawn, but if the low osmotic pressures of these tubule fluids be attributed to an active reabsorption of chloride and the high osmotic pressures of the bladder urines to a reabsorption of pure water (as distinct from fluid), then it would appear that the site of chloride reabsorption must be proximal to that of water. It has been customary to think that the site of water reabsorption, and of the increased osmotic pressure consequent upon it, lies in the loop of Henle. All three of these fluids had traversed the loop of Henle and yet did not show any increase in osmotic pressure. Two of them (expts. 57 and 59) may have traversed this loop with abnormal rapidity, but insofar as they permit a suggestion it must be that the site of water reabsorption is in the late distal tubule or even in the post-distal connecting tubule rather than in the loop of Henle.

Chloride and sodium. Since the fluid remaining in the proximal tubule is, as has been stated, in osmotic equilibrium with blood plasma, it had been anticipated that the chloride concentration of the two fluids would be the same. On the contrary, proximal tubule fluid proved to possess a distinctly higher chloride concentration than did plasma. This difference is demonstrated by the 14 experiments presented in table 1 and figure 3, and is supported by 3 additional experiments in which the site of collection was not accurately determined. It is interesting to recall that a similar difference between the chloride concentrations of proximal tubule fluid and plasma

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are oil and the cross-hatched sphere in drawings 5 and 6 is a droplet of mercury. Magnification 23×.

1. Hypertrophied guinea pig nephron from experiment 2. Site of collection, glomerular.

2. Normal guinea pig nephron from experiment 16. Site of collection 22 per cent down the proximal convolution.

3. Hypertrophied nephron from phlorhizinized rat. Two experiments, 18 and 40, were completed on the one tubule. Experiment 18, site of collection 24 per cent and experiment 40, 39 per cent down the proximal convolution.

4. Hypertrophied nephron from the phlorhinized rat of experiment 45. Site of collection 47 per cent down the proximal convolution.

5. Hypertrophied nephron from rat of experiment 35. Oil and mercury used to block the lumen. Site of collection 40 per cent down the proximal convolution.

6. Hypertrophied nephron from the rat of experiment 59. Oil and mercury used to block the lumen. Site of collection 25 per cent down the distal convolution.

was suspected in amphibia (8). There, the difference was so small in degree as to make its significance open to doubt. Here, it is altogether definite. Expressed as sodium chloride, the average concentration in tubule fluid is 0.870 per cent (0.7 to 0.986), the average in plasma 0.639 per cent (0.596 to 0.677). The fluid/plasma concentration ratio reaches an average of 1.40 in the first third of the proximal tubule and remains at that point without further increase throughout the second third of this segment. The most logical explanation[8] of these events is that chloride appears in glomerular fluid in concentrations appropriate to an ultrafiltrate of blood plasma, is concentrated in the early proximal by the reabsorption of a nearly chloride-free fluid, and is maintained at this concentration by the reabsorption in the later proximal tubule of a fluid containing approximately 1.4 times the chloride concentration of plasma.

The presence of a chloride concentration averaging 0.870 per cent in proximal tubule fluid is not, in itself, inconsistent with the recorded osmotic pressure determinations, for they indicated the fluid to be in equilibrium with blood plasma and therefore to possess a pressure close to that of a 1.0 per cent sodium chloride solution. It does, however, imply the absence of some other of the osmotically active constituents of plasma, unless the unlikely explanation of a depression of osmotic activity be considered. Ignoring this latter possibility, the most probable deficit would be in the bicarbonate ion. We have as yet no direct evidence that bicarbonate is reabsorbed and no quantitative determinations of pH have been made on tubule fluid; but the concentration of the sodium ion, in the two tubule fluids in which it was measured (table 1, expts. 12 and 26), was insufficient to cover in the one instance more than 40 per cent, in the other instance any, of the normal bicarbonate concentration of plasma in addition to the chloride demonstrably present in the tubule fluids. It may be observed that fluids of this general type, in osmotic equilibrium but electrolyte disequilibrium with blood plasma, are present elsewhere in the body, notably in the intestinal tract, eye, and central nervous system.

The majority of the bladder urine specimens in these experiments were hypotonic to blood plasma in respect to chloride. Some further portion of the nephron must therefore reverse the concentration ratio of the proximal segment and preferentially reabsorb the chloride ion.

[8] This explanation cannot be stated as a fact until the chloride concentration of glomerular fluid has been proven identical with that of plasma. This demonstration has been made in amphibia (9) but not in the present experiments. It is favored by observing that the 4 fluids with lowest concentration ratios were collected from the first third of the tubule. It is opposed by a single analysis of glomerular fluid (unlisted expt. B-46) which showed a fluid/plasma ratio of 1.40; this fluid was collected at the abnormally slow rate of 0.12 c.mm./hr. and we do not feel that its analysis deserves credence until more data are available.

SUMMARY

Occasional glomeruli, in guinea pigs, and certain portions of the proximal and distal tubules in a variety of mammals, are accessible to observation on the kidney surface during life. Fluid can be collected from these units in sufficient amounts to permit quantitative analysis and the precise site of the collection can be identified. A series of experiments are reported in which the composition of fluid thus collected has been compared with that of blood plasma in respect to protein, reducing substances before and after phlorhizin, exogenous creatinine, osmotic pressure, chloride and sodium.

The analyses indicate that glomerular fluid, entirely or nearly free of protein, contains reducing substances and creatinine in concentrations similar to those existing in plasma water. Within the proximal convolution, all of the reducing substances and at least two-thirds of the fluid are reabsorbed. This fluid reabsorption is an isosmotic process, accomplished without producing any increase in osmotic pressure of the fluid remaining within the tubule; it is not, however, a purely passive reabsorption of unchanged glomerular fluid for the chloride concentration of tubule fluid increases to a level 1.4 times that obtaining in blood plasma. The existence of this increased chloride concentration apparently requires that bicarbonate be preferentially reabsorbed by the proximal tubule, but the point has not yet been directly examined. The tentative conclusions, drawn from a small number of experiments with distal tubule fluid, are stated in the body of the paper.

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AN INVESTIGATION OF CHEMICAL TEMPERATURE REGULATION[1]

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Chemical temperature regulation may be defined as the increase in metabolic rate above the basal value when a resting and fasted animal is exposed to cold. There are two possible components of chemical temperature regulation, namely, 1, an increase of metabolism without shivering and presumably due to hormones which stimulate energy metabolism, and 2, shivering.

Cannon, Querido, Britton and Bright (1927) have stated that these two components of chemical temperature regulation constitute first and second lines of defense in the protection of the warm blooded animal against cold, with the hormonal mechanism forming the first line of defense and shivering the second. They have noted marked symptoms of sympathetic activity when cats with denervated hearts were cooled by intragastric ice water and observed a rise in metabolic rate of normal men without shivering when cooled in the same way. Since, as shown by Boothby and Sandiford (1923) and others, epinephrine injections increase metabolic rate it is logical to assume a hormone control of temperature regulation which functions to protect against cold. Swift (1932) observed an average increase of metabolic rate of men of 11.3 per cent when exposed to cold without shivering but these individuals experienced "increased muscle tension" which Swift considered to be part of the shivering mechanism. Swift could find no change in blood sugar during the exposure to cold and cited this as evidence opposing Cannon's theory. More recently Burton and Bronk (1937) have made similar investigations on anesthetized cats and have taken, as their criterion of shivering, action currents from the muscles. They found no increase in metabolism without evidence of muscular activity.

Recently we have concluded an extended series of observations on the effect of anesthesia by members of the barbital group on shivering. Bar-

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bital anesthetics are particularly depressant on temperature regulatory functions especially shivering. Having these trained dogs at our disposal we have investigated the change in oxygen consumption rate when normal trained dogs are slowly cooled and when shivering occurs. Dogs possess an advantage over human individuals in that they are not disturbed by subjective influences which in a man can effect shivering. For example, a human subject can voluntarily repress incipient shivering or can voluntarily imitate shivering movements. A trained dog not knowing the type of experimentation being observed is less likely to act voluntarily to aid or inhibit the start of shivering. The dogs were carefully trained and chosen and were used in a basal state without anesthesia.

It is quite obvious that when the two components of chemical temperature regulation are operating simultaneously in an extremely cold environment the two cannot be separated or evaluated. It is necessary to choose conditions wherein only one component is active. According to Cannon, Querido, Britton and Bright (1927) the hormonal component is first brought into play and is followed by shivering. Hence slow cooling of an animal should first initiate hormonal regulation to be followed later, when the cooling is more intense, by shivering. Rapid cooling might bring both factors into action simultaneously and separate effects could not be determined.

One of the most controversial phases of the problem is the measurement of shivering. This has been measured in three different ways as follows: 1, visual inspection; 2, mechanical shivering recorders (Swift, 1932, and Hemingway, 1940) and 3, measurement of muscle action currents (Burton and Bronk, 1937). Of these methods visual inspection is likely to be the least reliable and mechanical recorders which depend on limb movements may not reveal contractions of small muscle units or muscle tension changes. For these reasons the electrical method is the one of choice provided sufficiently high amplification can be obtained.

With these considerations in mind we have conducted experiments on dogs which were slowly cooled in an electrically shielded cooling cabinet. The onset of shivering was measured electrically, mechanically and visually and metabolic rate, temperature, respiratory and cardiac rate measurements were made under basal, cooling and shivering conditions.

Experimental. From twenty short haired dogs three were chosen for their ability to lie quietly in the metabolism apparatus for 2 to 4 hour periods. The animals were carefully trained for metabolism measurements and were used for the shivering experiments only when control experiments of two hours' duration showed uniform oxygen consumption rates. On the day of the experiment the fasting animal was brought to the metabolism room and required to lie quietly at rest for 2 to 3 hours. The dog was then placed in the apparatus, as shown in figure 1, with his head

sealed in a head mask through which air circulated from a metabolism apparatus. The body of the dog and head mask were placed in a double

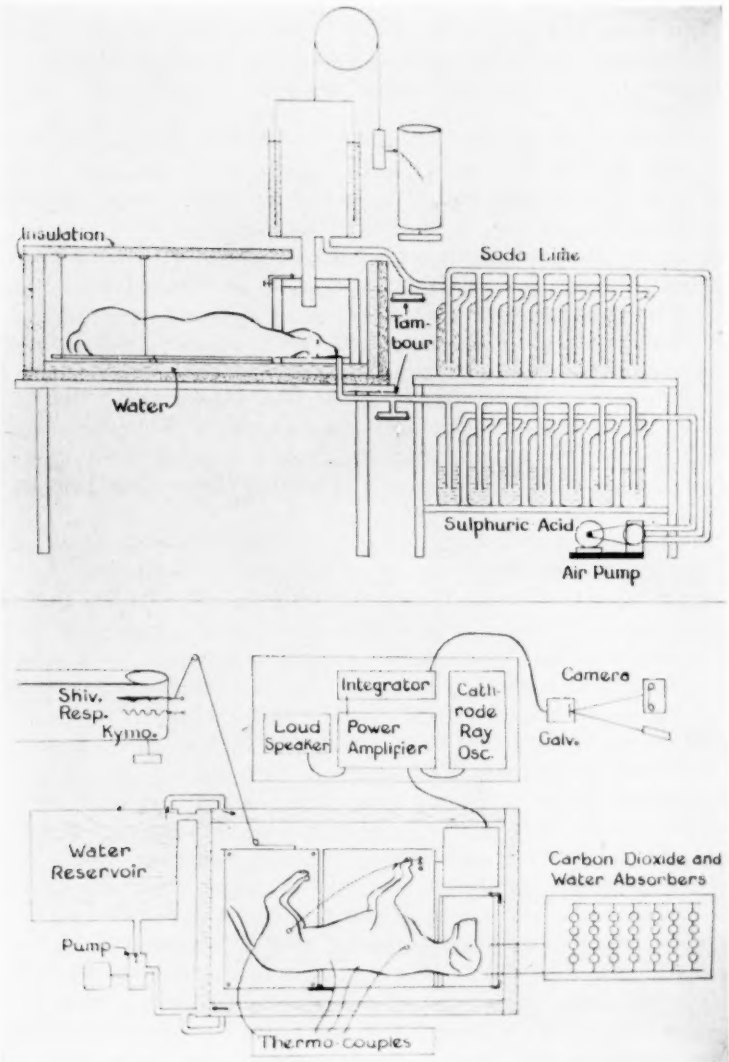

Fig. 1. Metabolism apparatus for measuring metabolic rate before and after onset of shivering.

walled sheet iron chamber which acted as an electrical shield. The temperature of the chamber was controlled by regulating the temperature of

water flowing from a reservoir through the hollow metal walls. In this way reproducible and finely adjusted cooling rates could be obtained. Rectal and skin temperatures were measured by thermocouples. The forepart of the dog including the thoracic region rested on a fixed platform while the hind legs rested on a movable platform suspended from wires and hinged to the fixed platform as shown in figure 1. Shivering was recorded by movements of the suspended platform. Small electrodes were placed on the skin above muscles which were chosen for their vigorous shivering movements when the animal shivered. These electrodes were connected to a preamplifier placed within the animal chamber and the preamplifier was connected with a power amplifier placed outside. Connected with the power amplifier there were a loud speaker, a cathode ray oscilloscope and an integrator. The integrator which served to rectify and summate the rapidly varying action potentials was connected to a recording galvanometer and produced records similar to that of figure 2. Oxygen consumption, carbon dioxide and respiratory water production

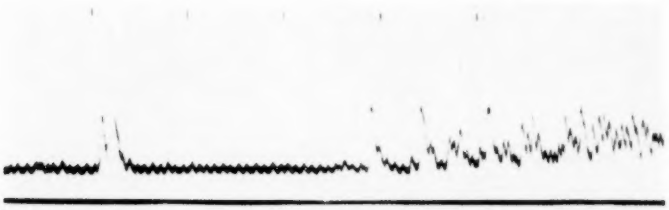

Fig. 2. Record of integrated action potentials showing abrupt onset of shivering. Time intervals one minute.

were measured by a closed circuit respiratory metabolism apparatus using soda lime and sulphuric acid absorbers. A refrigerator pump circulated air through the closed system and interchangeable sets of absorbers permitted metabolism measurements to be made in several periods.

The first experiments consisted of controls with the metabolism being measured in 20 to 30 minute periods during the course of 2 to 3 hours with the chamber temperature set at 28 to 30 degrees. In the course of these successive periods on any one day the variation of oxygen consumption rate was within ± 2 per cent although the day to day variation was greater. When shivering was to be produced the metabolism, in a control period at 28 to 30 degrees, was first measured. The absorption bottles were then changed and cool water was passed through the hollow walls of the chamber at such a rate that the chamber temperature dropped 5 to 6 degrees in 20 to 40 minutes. During this period designated as the cooling period all of the indicators were carefully watched for shivering which included the electrical measuring devices, the mechanical recorder and simply visual observation. At the first sign of shivering the absorption bottles were

again changed for the third or shivering period and the experiment was terminated after 15 to 30 minutes of shivering. Each entire experiment occupied four to five hours.

RESULTS. *The onset of shivering.* The onset of shivering was readily detected either by the cathode ray oscilloscope, the loud speaker or the integrator. Visible shivering and movements of the mechanical recorder usually followed within one minute. The onset of shivering was usually abrupt. In some cases short periods of shivering would be separated by quiscent intervals but as cooling continued the quiescent periods became progressively shorter in duration. There was no indication of a gradual

TABLE 1

Basal metabolic rates of dogs used in experiments compared with normal basal metabolic rates previously reported

Basal metabolism values

DOG	WEIGHT	SEX	OXYGEN CONSUMPTION RATE	CALORIES PER SQ. M. PER HOUR
	kilos		*liters per hour*	
A	9.3	F	2.89	28.2
B	13.0	M	4.40	34.3
C	7.5	F	3.56	39.7
Average				34.0

Normal basal metabolism values as given by other investigators

OBSERVERS	WEIGHT OF DOGS	CALS./SQ.M./HR.
Kitchen (1923)	10–15	40.3
Kunde (1926)	10–13	32.4
De Beer and Hjort (1938)	20–27	43.6
Lusk and DuBois (1924)		32.2
Morgulis (1924)	7–8	43.6
Average		38.4

rise in the integrated and rectified action potentials away from the base line before shivering commenced. The sudden onset of shivering is shown in figure 2.

Basal metabolism. The weight and sex of the three dogs used with their basal metabolism values are given in table 1. The heat production in calories per square meter per hour has been computed using Meeh's formula and assuming a value of 4.82 for the calorific value of a liter of oxygen. The basal metabolism values are in the range considered normal by the investigators listed in table 1. The dog A had an exceptionally low basal metabolic rate but this was to be expected because she was an old female, somewhat obese and very coöperative in her ability to rest in a relaxed state. The average basal metabolism of the three dogs, 34.0 calories per

square meter per hour, was below the average values which have been given by various investigators as normal values.

Rise in metabolic rate as a result of cooling without shivering. In table 2 are listed the relative values of the oxygen consumption rate of the pre-shivering cooling period followed by the shivering period. The control period (basal) value is arbitrarily assigned the comparative value of 100. There was always a slight rise in oxygen consumption rate which varied from 0 to +22 per cent with averages for each dog as shown in table 2. The averaged values varied from +5 to +10 per cent. When shivering started the increases of metabolic rate were much more pronounced and of course would have increased with further cooling.

TABLE 2

Increase of metabolic rate on cooling

DOG	SEX	NUMBER OF EXPERIMENTS	BASAL		COOL		SHIVERING	
			O_2	R.Q.	O_2	R.Q.	O_2	R.Q.
A	F	6	100	0.88	106	0.87	142	0.80
B	M	7	100	0.82	110	0.87	128	0.94
C	F	6	100	0.83	105	0.86	124	0.86
Average		19	100	0.84	107	0.87	130	0.89
Control		10	100	0.82	98	0.82	101	0.84

DISCUSSION. *Rôle of two components of chemical temperature regulation.* It is evident from the results obtained in table 2 that the non-shivering component of chemical temperature regulation is so small that it is practically without significance when an animal is exposed to cold under the conditions described. An increase of basal metabolic rate of less than 10 per cent can contribute very little in defense against cold and is unimportant when compared with the increases of metabolism due to voluntary movements and shivering. It is possible that the non-shivering component of chemical temperature is even less than that measured since the skin electrodes may not have picked up action currents from individual muscle fibers which contracted before larger muscle fiber groups became active. On the other hand the abrupt onset of shivering as shown in figure 2 is evidence against this since if shivering commences by a progressively increasing number of fibers becoming active then one could expect a slowly rising integrated action potential instead of the abrupt rise seen in figure 2. Hence it may be concluded that within the limitations of experimentation the results indicate that a non-shivering component of chemical temperature regulation exists but is without practical significance in protecting against exposure to cold.

The increase of basal metabolic rate which occurs after a prolonged exposure to cold has been observed by many investigators, e.g., Horvath,

Hitchcock and Hartman (1938), Horst, Mendel and Benedict (1933) and Gelineo (1934). This effect which may explain the seasonal variation of basal metabolic rate is evidently acquired as a result of acclimatization to a cold environment and requires a considerable period of time for its development. If an animal is exposed to cold without acclimatization, the non-shivering component of chemical temperature regulation is without appreciable value and the protection against cold depends practically on shivering. These results are suggestive of a general plan for temperature control of warm blooded animals in which a nervous mechanism (shivering) protects against a sudden exposure to cold whereas the hormone mechanism (thyroid-adrenal) functions as a result of prolonged exposure to cold and may explain acclimatization, see Horvath, Hitchcock and Hartman (1938).

SUMMARY

The oxygen consumption rate and CO_2 production of three trained dogs have been measured while the animals were slowly cooled in an electrically shielded metabolism chamber. The onset of shivering was noted by electrical, mechanical and visual methods. In the electrical method the action currents were picked up by small skin electrodes placed over shivering muscles. The action potentials were amplified and made to operate a loud speaker, a cathode ray oscilloscope and a recording electronic integrator. On cooling before shivering started there was an average increase of metabolic rate of 7 per cent over basal. During the first 20 minutes of shivering the increase over basal was 30 per cent. These results indicate that the increase of metabolic rate without shivering has little effect in combating exposure to cold.

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THE EFFECT OF MAGNESIUM DEFICIENCY ON THE EXCITABILITY OF THE VAGO-INSULIN AND SYMPATHETICO-ADRENAL SYSTEMS[1]

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In a series of papers Gellhorn and his collaborators, Cortell, Feldman and Kessler, have developed methods which allow one to investigate the excitability of the autonomic centers regulating the secretion of adrenalin and insulin in the rat. It was shown that drugs such as metrazol produce a hyperglycemia in the normal rat and a hypoglycemia in the adreno-demedullated rat. If, however, the drug is injected in the adreno-demedullated-vagotomized rat the blood sugar remains unchanged. These experiments were interpreted to mean that the drug acts on both sympathetico-adrenal and vago-insulin systems and that, in the normal animal, the former predominates over the latter. That metrazol, as well as other procedures, such as the administration of a convulsive shock to rats, give a quantitative estimate of the excitability of the autonomic systems was shown in studies on the effect of the thyroid hormone. It could be shown that the thyroidectomy lowered the excitability of the centers of the sympathetico-adrenal system without affecting the vago-insulin system. The injection of thyroxin increased the excitability of the sympathetico-adrenal system, but did not alter the vago-insulin system.

The present paper is an attempt to study the effect of variations in the ionic balance of the body on the excitability of the autonomic centers. From the studies of Kruse, Orent and McCollum, as well as of Greenberg and collaborators, it is known that Mg deficiency causes disturbances in the function of the central nervous system indicated by vasodilatation, increased irritability, and tonic-clonic convulsions. Greenberg observed that the susceptibility of Mg deficient rats to picrotoxin convulsions is greater than that of normal rats. Since earlier observations showed that picrotoxin acts similarly to metrazol these studies suggest that Mg deficiency alters the excitability of autonomic centers. The experiments reported in this paper show, indeed, that that is the case.

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METHOD. A diet similar to that used by Greenberg and collaborators was chosen. We used, however, adult rats and subjected them to a much milder degree of Mg deficiency than was used by the previously named authors. Correspondingly, the symptoms were very slight. The only constant effect of the diet given over several weeks was a roughing of the fur, and a loss in weight. The experiments were carried out on vagotomized and on adreno-demedullated rats. Fifty-five milligrams of metrazol per kilo was injected subcutaneously. An electric shock was applied to the head for 0.5 second using a General Electric Variac supplied with the 60 c.p.s. current as source of stimulation (cf. Kessler and Gellhorn). The blood sugar was determined with the Somogyi modification of the Shaffer-Hartman method.

RESULTS. The experiments were performed on two groups of rats, the first being kept on a diet containing 5.3 magnesium/100 grams food; the second on a similar diet containing only 3.7 mg magnesium/100 grams food.

Table 1 shows that vagotomized rats kept on these diets for one month show a greatly increased hyperglycemia on injection of metrazol. The differences between the vagotomized animals kept on control diet and the Mg deficient rats are statistically significant. The effect of the two diets is practically identical. Since, in the vagotomized rats, the rise in sugar is due exclusively to the liberation of adrenalin through the sympathetic system, the experiments seem to indicate that the centers of the sympathetico-adrenal system are in a state of a greatly increased excitability when the Mg content of the diet is reduced.

In contradistinction to these results, table 1 shows that adreno-demedullated rats do not show any significant changes in the blood sugar when injected with metrazol, although the rats on a control diet show regularly a fall in blood sugar under these conditions. Since the hypoglycemia in adreno-demedullated rats kept on a control diet and subjected to metrazol is due to the liberation of insulin through discharges mediated by the vagus (Feldman, Cortell and Gellhorn), the experiments suggest that the Mg deficiency of the diet caused a marked reduction in the excitability of the vago-insulin system.

Since it has been found previously that thyroidectomy distinctly lowers the excitability of the centers of the sympathetico-adrenal system, it was thought to be of interest to investigate whether this effect could be overcome by Mg deficiency. Consequently, thryoidectomized, vagotomized rats were kept on a Mg deficient diet and then tested with metrazol. Table 1 shows that the Mg deficiency displays its characteristic effect (increased hyperglycemic reaction to metrazol) in spite of the absence of the thyroid gland. The hyperglycemic reaction is, however, not quite as large as was observed in the Mg deficient animals in which the thyroid was intact.

An effort was made to determine whether similar results would be ob-

tained if the excitability of the autonomic centers were tested with the electroshock method. This procedure was applied on vagotomized and on

TABLE 1

Effect of metrazol (55 mgm/kgm.) on blood sugar of Mg-deficient rats

I. Vagotomized rats

BLOOD SUGAR, MGM. PER CENT	CONTROLS,* MEAN	I*					II†				
		1	2	3	4	Mean	1	2	3	4	Mean
minutes											
0	74.2±2.0	78.5	80.6	77.4	75.2	77.9±1.9	77.7	79.5	81.1	78.5	79.2±1.3
15	95.9±3.4	99.9	95.6	98.9	93.5	97.0±2.6	101.0	96.8	94.6	95.6	97.0±2.5
30	112.4±3.8	126.8	122.5	131.1	124.7	126.3±3.2	129.0	135.5	139.7	127.9	133.0±4.7
45	123.1±5.8	150.5	142.9	146.2	146.2	146.5±2.7	151.3	150.5	150.5	141.9	148.6±3.9
60	103.6±4.1	140.8	133.9	135.5	130.1	135.1±3.8	138.6	145.1	142.9	135.5	140.5±3.7

II. Adrenodemedullated rats

BLOOD SUGAR, MGM. PER CENT	CONTROLS,* MEAN	I‡					II§				
		1	2	3	4	Mean	1	2	3	4	Mean
minutes											
0	61.8±3.2	68.8	64.5	67.7	68.8	67.5±1.8	66.6	68.8	64.4	69.8	67.4±2.1
15	50.6±3.9	69.8	68.8	70.9	68.8	69.6±0.9	68.8	66.6	75.2	79.5	72.3±5.1
30	49.4±4.5	66.6	75.2	79.5	83.8	76.3±6.4	84.9	76.3	68.8	78.5	77.1±5.8
45		60.2	72.0	64.5	78.5	68.8±7.0	74.1	76.3	60.2	70.9	70.4±6.2
60	56.0±2.5	64.5	67.7	67.7	70.9	67.7±2.3	69.8	67.7	64.4	68.8	67.7±2.0

III. Thyroidectomized-vagotomized rats§

BLOOD SUGAR, MGM. PER CENT	CONTROLS,* MEAN	II§						
		1	2	3	4	5	6	Mean
minutes								
0	74.1±1.2	77.4	79.5	77.4	76.3	78.5	76.3	77.6±1.1
15	92.6±4.7	91.3	98.9	95.6	92.5	92.5	96.8	94.6±2.7
30	102.2±4.1	126.8	118.2	116.1	122.5	124.7	131.1	123.2±5.1
45	104.7±4.8		137.6	136.5	134.4	138.6	146.2	138.4±4.0
60	96.0±4.6		126.8	129.0	124.7		136.5	129.2±4.4

* Controls: Rats on normal diet.
On diet I (Mg content 5.3 mgm/100 grams) for 29 days.
† On diet II (Mg content 3.7 mgm./100 grams) for 29 days.
‡ On diet I for 26 days.
§ On diet II for 28 days.

adreno-demedullated rats subjected to the Mg deficient diet and the results thus obtained were compared with those observed on animals kept under control conditions. Table 2 shows the results, which are very similar to

those obtained in the experiments with metrazol. The hyperglycemic reaction following the electric shock in vagotomized and Mg deficient animals is significantly increased over and above that of the control animals. On the other hand, it is found that adreno-demedullated Mg deficient rats failed to show a fall in blood sugar after administration of the electric shock, although animals on a control diet show this effect regularly.

After these experiments had been completed the animals were returned to a normal diet and kept on this diet up to 62 days. During that time they were tested several times with metrazol, as shown in table 3. The return to the control diet had only a very slight, if any, effect on the ap-

TABLE 2

Effect of electrical shock (0.5 sec.) on the blood sugar of Mg-deficient rats

I. Vagotomized rats

BLOOD SUGAR, MGM. PER CENT	CONTROLS, MEAN	I*					II†				
		1	2	3	4	Mean	1	2	3	4	Mean
minutes											
0	77.4±1.4	75.2	78.5	80.6	78.5	78.2±1.9	79.5	77.4	80.6	76.3	78.5±1.7
10	97.1±1.7	99.9	101.0	96.8	97.8	98.9±1.7	98.9	101.0	99.9	96.8	99.2±1.5
40	119.3±2.0	127.9	130.1	124.7	123.6	126.6±2.6	126.8	132.2	127.9	125.7	128.2±5.1
70	106.2±4.0	122.5	120.4	118.2	117.1	119.6±2.1	121.5	124.7	116.1	117.1	119.9±3.5

II. Adreno-demedullated rats

BLOOD SUGAR, MGM. PER CENT	CONTROLS, MEAN	I*				II†				
		1	2	3	Mean	1	2	3	4	Mean
minutes										
0	63.4±2.1	64.5	68.8	66.6	66.6±1.8	68.8	66.6	66.6	67.7	67.4±0.9
10	55.5±3.5	62.3	67.7	63.4	64.5±2.3	64.5	66.6	62.3	66.6	65.0±1.8
40	43.5±5.6	62.3	70.9	61.2	63.8±4.4	60.2	67.7	68.8	69.8	66.6±3.8
70	64.4±4.0	62.3	68.8	64.5	65.2±2.7	65.5	68.8	72.0	66.6	68.2±2.5

* On diet I (Mg content 5.3 mgm./100 grams) for 33 days.

† On diet II (Mg content 3.7 mgm./100 grams) for 36 days.

pearance of the rats. The experiments with metrazol showed that even after 62 days the results were practically identical with those obtained at the end of the Mg deficiency period. Similarly, it was found, as indicated by the tests performed on the adreno-demedullated rats, that metrazol failed to elicit any hypoglycemia in these animals one or two months after they had been returned to the control diet.

It was tentatively assumed that the effect of Mg deficiency on the sympathetico-adrenal and vago-insulin systems of the rat was due to an alteration in the excitability of the centers of these systems. It was, however, not ruled out by the experiments described thus far that the amount of adrenalin and insulin secreted in the vagotomized and adrenodemedullated

TABLE 3

Effect of metrazol on blood sugar (mgm. per cent) of magnesium deficient rats returned to control diet

A. Adrenodemedullated rats

TIME	ON CONTROL DIET FOR										
	14 days*				30 days*	62 days*	21 days†				
	1	2	3	Mean, standard deviation	1	1	1	2	3	4	Mean, standard deviation
minutes											
0	70.9	68.8	67.7	*69.1* ± 1.3	68.8	66.6	68.8	65.5	66.6	68.8	*67.4* ± 1.4
15	68.8	67.7	64.5	*67.0* ± 1.8	83.8	68.8	74.1	64.4	68.8	68.8	*69.0* ± 3.4
30	67.7	67.7	65.5	*66.9* ± 1.0	79.5	74.1	72.0	60.2	72.0	75.2	*69.8* ± 5.7
45	64.5	66.6		*65.5* ± 1.1	70.9	70.9	72.0	66.6	70.9	70.9	*70.1* ± 2.1
60	67.7	68.8		*68.2* ± 1.7	66.6	70.9	69.8	65.5	66.6	69.8	*67.9* ± 1.9

B. Vagotomized rats

TIME	ON CONTROL DIET FOR													
	14 days*					30 days*				62 days*				21 days†
	1	2	3	4	Mean, standard deviation	1	2	3	Mean, standard deviation	1	2	3	Mean, standard deviation	1
minutes														
0	77.4	80.6	79.5	78.5	*79.0* ± 1.2	77.7	78.5	76.3	*77.5* ± 0.9	74.1	77.4	78.5	*76.7* ± 1.9	77.4
15	99.9	103.2	96.8	98.9	*99.7* ± 2.3	94.5	93.5	96.8	*94.9* ± 1.4	99.9	101.0	96.8	*99.2* ± 1.8	101.0
30	118.2	124.7	130.1	129.0	*125.5* ± 4.7	120.4	127.9	126.8	*125.0* ± 3.3	129.0	137.6	133.9	*133.5* ± 3.5	133.9
45	151.3	149.4	155.8	149.4	*151.5* ± 2.6	140.8	140.8	138.6	*140.0* ± 1.0	139.7	144.0	140.9	*141.5* ± 1.8	144.0
60	129.0	129.0	127.9	124.7	*127.7* ± 1.8	129.0	130.1	129.0	*129.4* ± 0.5	127.9	132.2	130.1	*130.1* ± 1.7	129.0

* Rats of group I.

† Rats of group II.

TABLE 4

Effect of adrenalin and insulin on the blood sugar of Mg-deficient rats

BLOOD SUGAR, MGM. PER CENT	CONTROLS, MEAN	I*				
		1	2	3	4	Mean
I. Adrenalin (1 cc. 1:25,000/kgm. intraperitoneally). Vagotomized rats						
minutes						
0	77.6 ± 1.3	79.5	81.1	77.4	78.5	79.1 ± 1.4
30	109.9 ± 3.0	110.9	107.5	118.2	105.3	111.2 ± 4.9
60	83.5 ± 2.5	87.0	86.0	84.9	89.2	86.8 ± 3.2
II. Insulin (0.01 unit/100 gram intraperitoneally). Adrenodemedullated rats						
0	66.8 ± 1.4	68.8	65.5	67.7	66.6	67.2 ± 1.2
60	43.7 ± 1.4	43.0	40.8	44.0	45.1	43.2 ± 1.6

* On diet I for 36 days.

rats respectively, might have been unaltered and that the significant changes in the response of the blood sugar to metrazol and electrical shock might be attributed to an alteration in the sensitivity of peripheral structures (liver, muscles) to insulin and adrenalin. Therefore, adrenalin was injected into vagotomized and insulin into adreno-demedullated rats, and the effect of the injection of these hormones on the blood sugar was tested on animals on control and on a Mg deficient diet. The experiments recorded in table 4 show conclusively that the Mg deficiency in the diet has no effect whatever on the hyperglycemic effect of adrenalin and on the hypoglycemic effect of insulin. It must, therefore, be concluded that the increased hyperglycemia in vagotomized rats is due to a sensitization of the center of the sympathetico-adrenal system. The absence of the hypoglycemic effect in adreno-demedullated rats indicates a decrease in the sensitivity of the center of the vago-insulin system. It is interesting to note that the decrease in the sensitivity of the centers of the vago-insulin system is apparently not restricted to this branch of the parasympathetic system. It was found that whereas normal rats subjected to a convulsive shock regularly urinated during the convulsions, the Mg deficient animals failed to do so. They show, however, during the period of catalepsy following the shock a decrease in pulse rate similar to that found in control animals.

SUMMARY

The effect of a magnesium deficiency in the diet on the centers regulating the discharge of adrenalin via the sympathetic system and the secretion of insulin through the vagus was studied in vagotomized and in adreno-demedullated rats. It was found that metrazol and electrically produced convulsive shocks produced a greater hyperglycemia in vagotomized magnesium deficient rats than in control animals. On the other hand, adreno-demedullated rats react to these tests with a distinct hypoglycemia, whereas magnesium deficient adreno-demedullated rats show no change in the blood sugar. Since magnesium deficiency does not alter the effect of adrenalin and insulin on the blood sugar, the experiments indicate a marked shift in the balance of the autonomic centers under the influence of magnesium deficiency. The centers of the sympathetico-adrenal system become greatly sensitized whereas the centers of the vago-insulin system fail to respond to stimuli which are effective in control animals. The effects persist for weeks after the animals have been returned to the control diet.

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THE MECHANISM OF THE COAGULANT ACTION OF DABOIA VENOM

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The coagulant power of the venom of the Daboia (Russell's viper) has been known for many years (1), but there have been numerous conflicting reports concerning the mechanism of its action. Lamb (1) found that whole blood or recalcified plasma was clotted by the venom in dilutions up to 1/1,000,000, whereas citrated plasma was clotted only by dilutions approximating 1/2,000 or stronger. The action of the venom *in vivo* was found to resemble that described by Wooldridge (2) for tissue extracts. Many subsequent investigators (3, 4, 5, 6, 7, 8) have verified the coagulant power of the venom for whole blood or recalcified plasma. The effect of the venom on citrated preparations has been disputed, some (4, 5, 6, 9) finding it to be coagulant, in high concentrations at least, while others (3, 8, 10, 11) obtained negative results. Arthus (3) observed that the venom did not behave either as a prothrombin or a thrombin, but resembled thromboplastin in its action. Houssay and Sordelli (4) demonstrated that the full coagulant effect of the venom required the presence not only of calcium ions but of thromboplastin and of serum (apparently as a source of prothrombin) as well. They concluded that the venom accelerated coagulation by "facilitating the formation of thrombin". Ganguly (8) found that the venom accelerated prothrombin conversion in the presence of platelets but not in their absence; he concluded that the venom hastened platelet lysis.

Eagle (11) studied the coagulant action of numerous snake venoms and offered the conclusion that coagulant venoms "are of two types; one which, like trypsin and like the calcium-platelet system, acts on prothrombin to form thrombin; and one which, like papain and thrombin, acts directly on fibrinogen to form fibrin." Eagle, employing only citrated plasma as a coagulating medium, did not detect any coagulant power in Daboia venom

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and did not pursue the possibility that it might represent a type of coagulant venom unlike those previously described. The work of Houssay and Sordelli foreshadowed this possibility, and the experiments presented herewith lend it further support.

MATERIALS AND METHODS. *Daboia venom.* Daboia venom was supplied[2] in 0.5 mgm. ampoules of dried material. Each ampoule was accompanied by a 5 cc. vial of 0.5 per cent phenol in distilled water, in which the usual dilution of 1/10,000 was made when the venom was to be used. Further dilutions of the venom were made in 0.9 per cent NaCl.

Plasma. Plasma for routine experiments was obtained from cows, by collecting blood in a paraffined pail containing enough 10 per cent sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) to make a final blood concentration of 0.6 to 0.9 per cent citrate. Less citrate not infrequently failed to prevent spontaneous coagulation. The blood was immediately chilled and centrifuged in a cold room for 1½ hours at 1200 to 1500 r.p.m. *Platelet-free plasma* was obtained by recentrifuging portions of the crude plasma twice more in the cold room for one hour at 2500 r.p.m., after which this clarified plasma was immediately passed through a Berkefeld "V" filter to remove the few remaining platelets.

For certain experiments, plasma was obtained from horses or from humans by venipuncture or from rabbits by cardiac puncture. These bloods were mixed at once with about 1/10 volume of 3.5 per cent sodium citrate, making a final citrate concentration of 0.35 to 0.4 per cent; in other respects the preparation followed that of bovine plasma.

Thromboplastic substances. Thromboplastic substances ("thromboplastins") of the following types were employed:

Human placental coagulant (12) was made up in fresh lots each week in this laboratory, lots T. G. 433 to 460 being used in the experiments described.

Bovine brain extract (prepared according to the method of Eley *et al.* (12)) was made up in two lots (T. G. 294 and 297) about two years previous to the experiments described and kept in the cold room until use.

Fresh purified cephalin, kept under alcohol until use, was prepared according to the method of Renall (13) and furnished through the kindness of Dr. H. C. Christensen of the department of biochemistry, Harvard Medical School. A two-year-old sample of cephalin (prepared according to Maltaner's modification (14) of the method of Levene (15)) was also employed, and a fresh, highly purified sample of lecithin (furnished by Dr. Christensen) was used as a control for the cephalin preparations.

Bovine platelets were obtained from the sediment of the recentrifuged plasma. They were washed by suspension in chilled citrated sodium chloride (1 part 3.5 per cent sodium citrate to 8 parts 0.9 per cent NaCl) and a centrifugation at 2500 r.p.m. They were then re-suspended in ci-

[2] Through the courtesy of Burroughs, Wellcome & Co., New York.

trated saline and centrifuged for 5 to 10 minutes at about 600 r.p.m. to sediment the red and white blood cells. This step was repeated several times until the supernatant contained not more than one red or white blood cell per 2,000 platelets. The platelet preparation was completed in not over six hours from the time of collecting the blood. The platelets so prepared showed but little disintegration in the next 48 hours and were very active in hastening coagulation.

Commercial coagulants. "Thromboplastin—Squibb", "Coagulen—Ciba", "Neo-Hemoplastin—Parke, Davis", and "Fibrogen—Merrell", were tested for their activity in comparison to the coagulants described above.

Fibrinogen. Fibrinogen was prepared by repeated precipitation (at 0–2°C. and pH 6.5) with $\frac{1}{3}$ volume of saturated ammonium sulfate. A 0.35 per cent concentration of sodium citrate was maintained in all reagents at all stages of the preparation. After three or four precipitations the fibrinogen was virtually free of prothrombin; it was then dissolved in 5 per cent NaCl containing 0.35 per cent sodium citrate and stored in a refrigerator until use. Suitable portions were diluted before use with 5 volumes of distilled water.

Prothrombin. Prothrombin was prepared by Green's modification (16) of Mellanby's method (17) and was kept in 0.9 per cent NaCl and 0.35 per cent sodium citrate in the refrigerator. A typical preparation of prothrombin, containing 0.59 mgm. N/cc., when converted to thrombin by 1 to 4 minutes' incubation with 0.1 per cent $CaCl_2$ and a trace of thromboplastin, would clot 10 volumes of citrated fibrinogen in 8 to 12 seconds at 37°C.

Calcium chloride. Calcium chloride was employed in a 2.5 per cent or (in some experiments) in a 1.1 per cent solution. The amount added was in all instances that which had been found to produce the most rapid coagulation of the preparation employed.

All experiments were carried out in 8 mm. glass flocculation tubes in a water bath at 37°C. Unless otherwise specified, each test tube contained one-half cubic centimeter of plasma, optimal $CaCl_2$ solution as stated above, such other reagents as are indicated in the protocols, and sufficient 0.9 per cent NaCl to bring the final volume up to one cubic centimeter. Clotting times were measured from the time of adding the last reagent ($CaCl_2$ or thrombin solutions) to the time when the contents of the tube ceased to flow on tilting.

RESULTS. *Action of Daboia venom on citrated and on recalcified preparations.* Daboia venom was added to citrated fibrinogen and to citrated plasma in concentrations of 0.03 mgm. per cc., and of 0.14 mgm. per cc. No evidence of coagulation was noted throughout the periods of observation. Control preparations were clotted rapidly by thrombin (see table 1).

Daboia venom, 0.01 mgm. per cc., was added to samples of recalcified

plasma from various animal species. Controls were clotted by recalcification alone. On addition of Daboia venom the various types of plasma clotted from 4 to 12 times faster than on recalcification alone (see table 2).

Action of Daboia venom on recalcified platelet-free plasma. Platelet-free plasma, prepared by Berkefeld filtration as described above, showed a recalcification time of from 1 to 12 hours, varying with different preparations. If Daboia venom (0.01 mgm.) was added before recalcification, the clotting time was reduced to 60 to 90 seconds. This acceleration was repeatedly observed, using different lots of plasma and varying amounts of Daboia venom. It was invariably found, however, that though the addition of venom greatly accelerated the coagulation rate of filtered plasma, the clotting time obtained was never as short as that produced by the venom in

TABLE 1

Action of Daboia venom on citrated fibrinogen and plasma

SUBSTRATE	COAGULANT			
	Daboia venom		Thrombin solution	
	Amount	Clotting time	Amount	Clotting time
	mgm.		*cc.*	*seconds*
Fibrinogen	0.03	No clot in 20 minutes	0.3	35
Horse plasma	0.03	No clot in 20 minutes	0.3	30
Horse plasma	0.14	No clot is 2 hours	0.15	60

TABLE 2

Action of Daboia venom on recalcified plasma

SOURCE OF PLASMA	CLOTTING TIME ON ADDITION OF:	
	$CaCl_2$	$CaCl_2$ + 0.01 mgm. Daboia venom
	minutes	*seconds*
Horse	6	30
Cow	7	70
Rabbit	2	29
Man	3	26

unfiltered (control) plasma. In this respect the action of Daboia venom differed from that of tissue extract, which clotted platelet-free plasma in essentially the same time as it clotted the unfiltered control. Typical results are summarized in table 3.

Inability of Daboia venom to substitute for prothrombin. The observations of Arthus (3), of Houssay and Sordelli (4) and of Ganguly (8), and those presented above could be explained by the assumption that Daboia venom functions as a prothrombin, if it were further assumed that the reagents employed in these experiments were so nearly free of thromboplastic substances that the conditions for thrombin formation had not prevailed. This possibility was therefore tested as follows:

1. *Incubation of Daboia venom with tissue extract and calcium chloride.* One one-hundredth milligram Daboia venom in 1 cc., plus 0.25 mgm. T. G.

294 and 0.1 cc. of 1.1 per cent $CaCl_2$ solution, incubated at 37°C., developed no power to clot fibrinogen during $1\frac{1}{2}$ hours of observation.

Control: one cubic centimeter prothrombin solution, plus T. G. 294 and $CaCl_2$ solution as above, after 1 minute and 41 seconds' incubation developed the power to clot 5 volumes of fibrinogen in 9 seconds.

2. *Influence of adding Daboia venom to prothrombin: Rate of thrombin formation and potency of thrombin formed.* The possibility existed that

TABLE 3

Action of Daboia venom on recalcified Berkefeld-filtered (platelet-free) plasma and on unfiltered plasma; comparison with tissue extract

PLASMA	CLOTTING TIME ON ADDITION OF:		
	$CaCl_2$	$CaCl_2$ + 0.01 mgm. Daboia Venom	$CaCl_2$ + optimal tissue extract (prothrombin time)
	minutes	*seconds*	*seconds*
Bovine: unfiltered	7	70	33
Bovine: filtered	Over-night	170	34
Equine: unfiltered	6	30	33
Equine: filtered	60	75	32

TABLE 4

Influence of Daboia venom on conversion rate of prothrombin and on potency of thrombin formed; comparison with tissue extract

COAGULANT 1 cc. PROTHROMBIN + 0.2 cc. OF 1.1% $CaCl_2$ +	MAXIMUM POTENCY THROMBIN MIXTURE CLOTTED FIBRINOGEN IN*	CONVERSION TIME MAXIMAL POTENCY FIRST REACHED IN
	minutes	*minutes*
(No coagulant added)	6†	20†
	seconds	
Daboia venom, 0.001 mgm.	11	30
Daboia venom, 0.01 mgm.	9	20
Daboia venom, 0.03 mgm.	9	9
T. G. 294, 0.015 mgm.	11	4
T. G. 294, 0.25 mgm.	9	$1\frac{3}{4}$
T. G. 294, 0.015 mgm. + Daboia venom, 0.01 mgm.	10	2

* One-tenth cubic centimeter of thrombin mixture added to 0.5 cc. of fibrinogen.

† Maximal potency not obtained during period of observation.

Daboia venom could function as a prothrombin only in the presence of natural prothrombin. To test this possibility, varying amounts of tissue extract and of the venom were incubated with prothrombin and $CaCl_2$ and the potency of the thrombin formed and the time required to develop a given potency were noted. The results, presented in table 4, indicated that the venom—like tissue extract—accelerated the formation of thrombin but did not significantly affect the maximum amount of thrombin obtainable.

3. *Daboia venom as a substitute for prothrombin in plasma with a subnormal prothrombin content.* To test the possibility that Daboia venom might function as a prothrombin substitute only in the presence of the normal constituents of plasma, the action of the venom was observed on plasma preparations containing varying percentages of the normal prothrombin. These were prepared by mixing appropriate proportions of Berkefeld-filtered plasma with plasma rendered virtually prothrombin-free by alumina absorption (18). The "prothrombin time" (19) for each mixture was determined by the addition of the optimal amount of tissue extract and $CaCl_2$ solution. Daboia venom (0.01 mgm.) was then added to samples of each mixture; tissue extract and $CaCl_2$ were added as before, and the clotting time noted (see table 5). It was found that 0.01 mgm. Daboia

TABLE 5

Coagulant action of Daboia venom on plasmas with varied prothrombin concentration

PROTHROMBIN CONCENTRATION	CLOTTING TIME ON ADDITION OF $CaCl_2$ AND:	
	Optimal tissue extract (prothrombin time)	0.01 mgm. Daboia venom + optimal tissue extract
per cent	*seconds*	*seconds*
100	41	18
80	42	21
60	56	24
40	79	30
20	110	43
±0.25	*	420

* Incomplete clotting in 20 to 30 minutes.

TABLE 6

Coagulant action of equivalent quantities of Daboia venom and of tissue extract on recalcified Berkefeld-filtered bovine plasma

UNITS	CLOTTING TIME	
	Daboia venom, 1 unit = 0.001 mgm.	T. G., 297, 1 unit = 0.4 mgm.
	seconds	*seconds*
1	211	179
3	123	130
5	108	100
7	93	93
10	88	85

venom added to the 20 per cent-prothrombin mixture gave a clotting time equivalent to that of the 80 per cent-prothrombin mixture; here the Daboia venom appeared to be equal in effect to 60 per cent of the normal prothrombin content of the plasma. The same amount of venom, however, in the presence of 0.25 per cent of the normal prothrombin, did not produce the effect of even 20 per cent of the normal prothrombin.

Quantitative effect of Daboia venom on coagulation rate. Dilutions of tissue extract and of Daboia venom having equivalent potencies were prepared, and multiples of these equivalent units of each substance were compared for their power to clot recalcified Berkefeld-filtered bovine plasma. A typical series of determinations is given in table 6. In this and similar experiments it was observed that the amount of Daboia venom added to plasma bore a roughly quantitative relationship to the coagulation rate

produced and, furthermore, that the results obtained were parallel to those observed when equivalent amounts of tissue extract were used. A mathematical statement of the relationship between tissue extract concentration and clotting time has been formulated by Mills (20) for the plasma-calcium-thromboplastin system. The data so far obtained for Daboia venom indicate a similar relationship.

Synergistic action of Daboia venom and thromboplastin. Although, as indicated above, given increments of either Daboia venom or tissue extract alone produced a proportionate acceleration of coagulation, this did not hold true when the two coagulants were employed in combination. The combined action of Daboia venom and tissue extract was not additive; it was synergistic. Typical results are presented in table 7. There it is apparent that the use of equivalent unit quantities (0.01 mgm. and 0.04 mgm. respectively) of Daboia venom and T. G. 294 in combination yielded a coagulation rate very much faster than that obtained by doubling the unit quantity of either coagulant alone.

TABLE 7

Coagulant action of equivalent amounts of tissue extract and of Daboia venom employed separately and in combination; recalcified Berkefeld-filtered bovine plasma

COAGULANT	CLOTTING TIME
	seconds
0.01 mgm. Daboia venom	82
0.04 mgm. T. G., 294	86
0.02 mgm. Daboia venom	71
0.08 mgm. T. G., 294	73
0.01 mgm. Daboia venom + 0.04 mgm. T. G., 294	30

The synergistic effect described above was observed when Daboia venom was used in combination with other thromboplastic substances (see table 8). Of particular interest was the finding that the synergistic interaction of the venom with purified cephalin was fully as marked as that observed with crude tissue extracts. That this did not represent a non-specific group reaction with phospholipids was shown by testing the interaction of the venom with lecithin. The very slight acceleration of coagulation observed on employing 4 mgm. of lecithin—comparable to that obtained with 0.002 mgm. of cephalin—may properly be ascribed to the unavoidable residual contamination of the lecithin with cephalin.

Interaction of Daboia venom and partially inactivated thromboplastic substances. It was found that the coagulant activity of certain tissue extract preparations could be partially destroyed by heat or by extraction with ether. Used in conjunction with Daboia venom, however, these extracts exhibited an activity nearly equal to that of the untreated extracts used with venom. A 2 year-old cephalin preparation, showing very little co-

agulant activity when used alone, likewise became quite as active as a fresh potent preparation when used in conjunction with the venom (see table 9).

TABLE 8

Synergistic action of Daboia venom used in combination with thromboplastic coagulants (tissue extracts, platelets, phospholipids, commercial coagulant extracts); recalcified Berkefeld-filtered bovine plasma

THROMBOPLASTIC COAGULANT		CLOTTING TIME		
Kind	Amount	Thromboplastic coagulant alone	Daboia venom (0.01 mgm.) alone*	Daboia venom + thromboplastic coagulant
		seconds	*seconds*	*seconds*
Cephalin	2 mgm.	510	84	15
Cephalin	0.002 mgm.		84	58
Lecithin	4 mgm.		84	61
Bovine platelets	5×10^5	360	60	26
Bovine platelets	7.5×10^6		63	47
Placental coagulant	2 mgm.	270	85	25
Fibrogen—Merrell	0.1 cc.	46	95	12
Thromboplastin—Squibb	0.1 cc.	1,200	165	14
Coagulen—Ciba	0.1 cc.	None in 75 minutes	165	19
Neo-Hemoplastin—Parke, Davis	0.1 cc.	None in 75 minutes	165	120

* Differences in coagulation time observed with Daboia venom alone are due to variations in different lots of plasma.

TABLE 9

Interaction of Daboia venom with fresh and with partially inactivated thromboplastic coagulants; recalcified Berkefeld-filtered bovine plasma

THROMBOPLASTIC COAGULANT		CLOTTING TIME ON ADDITION OF:	
Kind	Condition	Thromboplastic coagulant alone	Thromboplastic coagulant + 0.01 mgm. Daboia venom
		seconds	*seconds*
T. G., 294	Fresh	44	21
T. G., 294	Heated to 60°C., 1 hour	75	22
T. G., 449	Fresh	270	25
T. G., 449	Extracted with ether	1,400	31
Cephalin, Lot B	Fresh	360	16
Cephalin, Lot A	2 years old	1,800	17

Discussion. It has been shown that Daboia venom, in the presence of a sufficient concentration of calcium ions, is a highly potent coagulant with

an activity comparable to that of cephalin or tissue extract. Since this potent activity is dependent upon the presence of calcium, the action of the venom is distinct from that which has been demonstrated for papain or trypsin (21) and for the snake venoms which have been shown (11) to resemble those enzymes in their coagulant action. Furthermore, it has been shown above that the action of the venom does not depend on the presence of formed platelets, nor does the venom function as a prothrombin substitute. Moreover, it has been found that the venom, used in conjunction with any one of a variety of thromboplastic substances, exerts a synergistic action on clotting rate which has no parallel in the interactions of thromboplastic substances themselves. This finding is borne out by the data in table 3, where it it seen that the activity of tissue extract is not affected by the presence of platelets, whereas the contrary is true of the venom. Thus the venom cannot be regarded as identical with thromboplastin in its action.

The observations presented above entirely support the conclusion of Houssay and Sordelli (4) that the venom "facilitates the formation of thrombin." While the nature of this facilitating action is as yet undetermined, the data presented indicate that it consists of an "activation," or acceleration, of the function of thromboplastic substances in the conversion of prothrombin to thrombin. The interaction of the venom with cephalin is of particular significance, since cephalin is the only known chemical entity found in most (if not all) thromboplastic substances which manifests the coagulant activity of the parent substance, and is therefore widely believed to be the active principle of such substances. It may be suggested, therefore, that the coagulant action of Daboia venom involves a chemical or physical interaction with cephalin through which the interaction of cephalin with prothrombin is in turn facilitated.

SUMMARY

1. Daboia venom exerts a significant coagulant action on plasma only in the presence of a sufficient concentration of calcium ions.

2. The coagulant action of Daboia venom is independent of the presence of formed platelets.

3. The coagulant action of Daboia venom is synergistic with the action of tissue extracts, of platelets, and of cephalin.

4. In the presence of Daboia venom, the coagulant activity of certain partially inactivated thromboplastic substances is largely or wholly restored.

5. The coagulant mechanism of Daboia venom is distinct from that of thrombin, prothrombin, or thromboplastic substances and from trypsin, papain, or the snake venoms which have been shown to resemble those enzymes in their coagulant action.

6. The coagulant action of Daboia venom appears to be exerted through an interaction with tissue extracts or with the cephalin contained in such extracts.

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[3]Since this paper was submitted for publication, it has been learned that J. W. Trevan and R. G. McFarlane (Annual Report of the Medical Research Council, 1936–37, p. 143) observed an augmentation of the coagulant action of Daboia venom on mixing it with lecithin; and that others have confirmed this observation, including F. C. G. Hobson and L. J. Witts (Jour. Path. and Bact., **52,** 367, 1941) who have also demonstrated quantitatively that the action of the venom is dependent on the presence of calcium, and have indicated the influence of the presence of platelets.

THE EFFECT OF ADRENALECTOMY ON FAT ABSORPTION[1]

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The study of intestinal absorption has always presented one of the most interesting problems in physiology. Not only are the pancreas, the liver and the intestinal mucosa involved but also there is considerable evidence that the adrenal cortex may play a rôle.

The influence of the adrenals on fat absorption has been supported by the experiments of Verzar and Laszt (1935), who also postulated a somewhat similar mechanism for carbohydrate absorption (Judovits and Verzar, 1937). In an extensive series of investigations, these workers found that fat absorption was inhibited by adrenalectomy in rats and that the normal function could be restored by the administration of cortical extract.

In the absence of the adrenal cortex this effect was attributed to the failure of phosphorylation, which phenomenon is postulated as a prerequisite for the absorption of the fatty acid portion of the fat molecule. The results of Artom and Peretti (1935) using iodized fats and of Sinclair (1936) who employed elaidic acid indicate that the ingested fatty acids do become incorporated into the phospholipids of the intestinal mucosa. Not only would it appear that the adrenal glands regulate fat and carbohydrate absorption but the work of Clark (1939) and of Stein and Wertheimer (1941) would seem to indicate that this gland also controls the absorption of sodium chloride.

On the other hand, evidence from this laboratory (Deuel, Hallman, Murray and Samuels, 1937) indicates that the rôle of the adrenal cortex in carbohydrate absorption is a secondary one. The lowered absorption of glucose did not occur if dehydration and the consequent circulatory disturbances were avoided by administration of Rubin-Krick or sodium chloride solution following adrenalectomy. That a similar explanation for the lowering of fat absorption in adrenalectomized animals may obtain, is indicated by the report of Barnes, MacKay, Wick and Carne (1939) who found

[1] These data are from a thesis to be presented by Lucien Bavetta to the Graduate School of the University of Southern California in partial fulfilment for the degree of Doctor of Philosophy.

no effect on the rate of absorption of methyl esters of fatty acids or of corn oil itself in adrenalectomized rats and also by that of Barnes, Miller and Burr (1939) who used spectroscopically active fatty acids.

Because of these divergent results it seemed desirable to reinvestigate the effects of adrenalectomy on the absorption of fats. In this study a comparison has been made of the rate of absorption of hydrogenated cottonseed oil in normal, sham-operated, and adrenalectomized rats which were given either Rubin-Krick solution alone, water, or water and cortin.

METHODS. The experimental animals were female albino rats from our stock colony weighing 120 to 160 grams kept on our regular stock diet. They were adrenalectomized under ether anesthesia using the lumbar approach. Experiments on fat absorption were made 7 to 10 days after the operation. In most cases the completeness of operation was checked post mortem.

The procedure for the determination of the rate of fat absorption was similar to that employed earlier (Deuel, Hallman and Leonard, 1940) and the physical and chemical constants of the fat were identical with those of the hydrogenated cottonseed oil reported in our earlier work. A fasting period of 24 hours preceded the tests. The fat was administered at a level of 300 mgm. per 100 sq. cm. of body surface. The latter was calculated by the formula of Lee (1929). During the tests the animals were kept in separate cages and any experiments showing evidence of diarrhea were discarded. The fatty acids recovered from the gastro-intestinal contents were estimated by titration of the ether extract with 0.1 N NaOH using phenolphthalein after solution in petroleum ether and isopropyl alcohol. The milligrams of fatty acids were calculated from the titration by use of a fatty acid equivalent based on the saponification number of the fat.

Cortin[2] was administered in the drinking water for 4 days prior to the absorption tests in amounts of 1 cc. daily. This amount of hormone was added each day to approximately the volume of water taken by the rat the previous day. One hour before the fat feeding, each animal was given an additional 0.5 cc. of cortin by stomach tube.

RESULTS. A summary table showing the rate of absorption of normal and adrenalectomized rats is recorded in table 1 while the control tests on the fasted animals are given in table 2.

The fat absorbed is calculated from the difference between the amount fed and the corrected amount recovered from the gut. The quantity actually recovered from the gut is first corrected by subtraction of the amount of ether-soluble material which was removable from the gastro-intestinal tracts of rats fasted for a similar period but fed no fat (table 2). A further correction is applied for the extent of recovery based on the values obtained

[2] Cortin was kindly furnished for these studies by Dr. E. C. Kendall of the Mayo Clinic who suggested the dosage and method of administration.

when known amounts of fat are given and the gastro-intestinal tract removed immediately. The latter value employed was for a 93.6 per cent recovery.

TABLE 1

Summary table showing the fat absorbed in three hour period by female rats fasted one day and fed 300 mgm. of hydrogenated cottonseed oil per 100 sq. cm. of surface area

EXPERIMENTAL CONDITION	NUMBER OF EXPERIMENTS	AVERAGE WEIGHT	AVERAGE SURFACE AREA	FAT ABSORBED IN MGM. PER HOUR*			TITRATION OF ETHER EXTRACT	FATTY ACID IN ETHER EXTRACT		NEUTRAL FAT HYDROLYZED PER HOUR*
				Per 100 gm.	Per 100 sq. cm.			Total	Per cent of total lipid	
					Total	M.D. ÷ S.E.M.D.†				
		grams	*sq. cm.*				*cc. 0.1 N NaOH*	*mgm.*		*mgm. per 100 sq. cm.*
Normal (a)	17	117	219	68.2±1.8	36.3±1.0		3.0±0.3	83.3	24.5	46.2±1.5
Normal—Sham-operated (b)	16	125	227	63.3±2.3	34.9±1.0		2.4±0.3	66.5	15.6	46.0±1.7
Adrenalectomized—Rubin-Krick (c)	31	146	248	46.6±2.9	27.6±1.6	4.53(a) 3.88(b)	7.9±0.2	217.0	39.9	54.1±2.4
Adrenalectomized—Water only (d)	6	141	244	38.3±6.5	22.7±3.7	3.57(a) 3.19(b)	6.3±0.6	170.0	30.3	45.7±4.5
Adrenalectomized—Cortin (e)	20	127	229	71.7±3.7	39.7±2.1	4.25(c) 4.00(d)	1.7±0.3	47.1	12.9	47.7±2.7

* Including standard error of mean = $\sqrt{\frac{\Sigma d^2}{n}} \Big/ \sqrt{n}$

d = deviation from mean
n = number of observations

† Mean difference: standard error of mean difference. When this value exceeds 3.0 the results are considered significant. The letter in parentheses indicates groups with which comparisons are made.

TABLE 2

Summary table showing ether-soluble material in female rats fasted one day without fat feeding

EXPERIMENTAL CONDITION	NUMBER OF EXPERIMENTS	AVERAGE WEIGHT	TOTAL FAT IN GUT*	TITRATION OF ETHER EXTRACT	ETHER SOLUBLE EXTRACT AS FATTY ACID*
		grams	*mgm.*	*cc. 0.1 N NaOH*	*mgm.*
Normal	8	127	26.6±2.1	0.88	18.5±4.6
Sham-operated	14	105	34.0±3.5	0.42	11.7±1.3
Adrenalectomized—Rubin-Krick	19	161	28.0±3.6	0.53	14.6±1.8

* Including standard error of mean calculated as in table 1.

The neutral fat hydrolyzed was calculated to determine the comparative lipolytic action in the different groups. The total neutral fat hydrolyzed was the sum of the amount of fat absorbed plus the amount of the hydro-

lyzed fat left in the gut. The latter was determined by titration and expressed as neutral fat by multiplication of the titration value (corrected for fasting controls) by the saponification equivalent.

DISCUSSION. There is a decrease of approximately 38 per cent in the rate of fat absorption in the untreated adrenalectomized rats as compared with normals. When salt solution is administered to operated animals, the depression is somewhat less (24 per cent) but the difference is still highly significant from a statistical standpoint. Although the rate of absorption of normal rats was slightly lower on an average after undergoing a sham operation, it was significantly higher than that of the adrenalectomized animals. That the depression is to be ascribed to the absence of the adrenal cortex is evident from the fact that absorption could be completely restored by the administration of cortin. This fact is corroborated by the observation that the intestinal lymphatics appeared practically white in the normal and cortin-treated rats killed at the height of fat absorption while this was not evident in the adrenalectomized animals to which cortin was not given.

The decreased absorption apparently results from a failure to remove fatty acid at a normal rate as evidenced by greater accumulation of free fatty acids in the gut contents of the adrenalectomized animals not receiving cortin. On the other hand, there is no evidence of any decrease in lipolytic activity associated with the removal of the adrenal gland. These results would seem to support the theory of Verzar and Laszt (1935), although they do not give any evidence as to whether phosphorylation is involved.

SUMMARY

A definite inhibition in fat absorption was noted in adrenalectomized rats. It was only slightly improved by the administration of Rubin-Krick solution. After adrenalectomy larger amounts of fatty acids accumulated in the intestine than occurred normally. Both of these phenomena were restored to normal by the administration of cortin.

There is no evidence that adrenalectomy alters lipolytic activity.

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FACTORS INFLUENCING THE EXCRETION OF UROGASTRONE[1]

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The fact that urogastrone (1) extracted from urine, and enterogastrone (2) extracted from the mucosa of the small intestine, exert somewhat similar inhibitory effects on gastric secretion and motility, suggested the possibility that urogastrone may represent enterogastrone, which has been eliminated from the body by the kidneys. It is known that enterogastrone is formed by the mucosa of the small intestine and that ingestion of neutral fat is especially effective in causing its liberation (3). Accordingly, if urogastrone is the same as enterogastrone, the feeding of fat should augment its output in the urine and the removal of the small intestine should eliminate it from the urine. Neither duodenectomy nor gastrectomy eliminates urogastrone from the urine, according to Friedman *et al.* (4), but this evidence is incomplete, since enterogastrone is formed by the entire small intestine and not by the stomach. It was the purpose of the present investigation to determine the effects of complete enterectomy and the feeding of fat on the output of urogastrone. In addition, the output in patients with peptic ulcer was investigated, since Friedman *et al.* (5) state that active extracts can be prepared from the urine of ulcer patients, whereas Necheles (6) has claimed that ulcer urine contains less activity than normal urine.

METHODS. The general plan of the experiments was as follows: In order to determine the effect of removal of the small intestine, the output of urogastrone during fasting was compared with the output after complete enterectomy. The results were controlled by similarly comparing the output before and after a control or "dummy" operation. In order to determine the effect of feeding fat, the output of urogastrone during fasting was compared with the output when a high fat diet was fed. The results were controlled by similarly comparing the output during fasting with the output when a low fat diet was fed.

A total of eleven series of experiments was performed, each of which included from 3 to 5 dogs. In each series the urine was pooled for purposes of extraction. In most of the series the urine was collected entirely by

[1] Aided in part by a grant from the Committee on Endocrinology of the National Research Council.

frequent catheterization, employing female dogs previously prepared surgically for this procedure.

Fasting urine was not collected until the dogs had been without food for two days, and was continued for approximately a week. In some series the animals were given fluid only parenterally; in others they were allowed to drink water *ad lib.*

The surgical procedures were as follows. *The enterectomy consisted of* a, removal of the small intestine from the pyloric sphincter to the cecum; b, external drainage of the stomach through a large Pezzer catheter sutured into the pyloric opening; c, ligation of the common bile duct, with external drainage of the gall bladder through a small Pezzer catheter, and d, removal of the body of the pancreas leaving the ligated head and tail. The enteroantrectomy was done similarly, except that the pyloric antrum was also removed. *The control or "dummy" operation consisted of* a, transection at the incisura angularis with blind closure of the distal stump; b, external gastrostomy as above; c, ligation of the common bile duct and cholecystostomy as above; d, ligation of the pancreatic ducts. This operation differed from the others only in that the small intestine, with digestive secretions excluded, was allowed to remain in the abdominal cavity.

Beginning approximately 12 hours after the operation, urine, bile and gastric juice were collected and fluid was administered subcutaneously at intervals throughout the day and night, for a period of approximately a week. The animals remained in good condition during this interval; 6 animals were maintained for 14 to 33 days by the intravenous administration of glucose and amino acids after urine collection was terminated.

Urine was collected from dogs over a period of a week or more, during which they were fed twice daily a high fat diet consisting of prepared dog food with 30 per cent added fat, or a low-fat diet consisting of the prepared dog food alone, which contained 2.5 per cent fat.

The effect of feeding a fatty meal was also investigated in 28 human subjects. Urine was collected on one fasting day and on another day when three meals of 30 per cent cream and crackers were taken. The body weight and the exact period of urine collection were recorded for each subject and the urine was pooled for purposes of extraction.

Urine was collected similarly from eight patients with duodenal ulcer over a period of several days while they were maintained on a milk diet.

The daily urine samples were extracted by the benzoic acid adsorption method previously described (1). The crude concentrates thus obtained were further purified by various methods. As new methods of purification were developed, they were applied to the dog urines. Consequently different methods were used in the different series, although without exception the same procedure was employed within any given series.

The extracts were assayed in either Heidenhain pouch dogs, or dogs with

pouches of the entire stomach (vagotomized). In these animals the milligrams of free acid secreted in response to histamine in the forenoon was compared with the response to the same dose of histamine given 3 hours later and 10 minutes after the intravenous injection of the extract to be assayed. From a previously established curve the average percentage inhibition was converted into "doses" of urogastrone. A "dose" is defined as the quantity which produces 50 per cent inhibition under the conditions of the assay. In order to avoid the implication of a high degree of accuracy, the term "dose" is used instead of "unit."

TABLE 1

The effect of surgical procedures on the output of urogastrone

SERIES	NUMBER OF DOGS	CONDITION	ASSAY							YIELDS					
			Number of assays	Dose			Effect			Per 100 cc. urine		Per 100 kgm. hrs.			
				Mgm.	Cc. urine	Kgm.-hrs.	Temp. rise	% inhib.	Inhib. doses	Extr.	"Doses"	Urine	Extr.	"Doses"	% change
							°F.			*mgm.*		*cc.*	*mgm.*		
1	3	Fasted + fluid	17		350	105	0.75	−34	0.76		0.22	360		0.72	
		Enterectomy	14		555	150	1.10	−31	0.66		0.12	336		0.44	−39
2	4	Fasted + fluid	12	5	393	205	1.35	−32	0.70	1.27	0.19	191	2.46	0.34	
		Enteroantrectomy	11	6	209	209	1.05	−14	0.26	2.81	0.12	100	2.81	0.12	−65
			12	9	188	207	1.25	−8	0.14	4.80	0.07	91	4.35	0.07	−80
3	4	Fasted	9	7	216	505		−44	1.08	3.24	0.49	43	1.38	0.21	
		Enterectomy	5	20	301	506		−40	0.94	6.65	0.30	60	4.00	0.19	−10
4	5	Enteroantrectomy	12	20	474	504	0.90	−30	0.64	4.22	0.14	94	3.96	0.13	−38*
1	4	Fasted + fluid	12	4	203	277	0.65	−35	0.80	1.87	0.39	77	1.44	0.29	
		Control operatn.	18	15	135	195	1.30	−53	1.40	11.13	1.00	70	7.76	0.72	+148
2	4	Fasted	7	5	167	391		−36	0.82	3.00	0.50	44	1.34	0.21	
		Control operatn.	10	13	273	308	0.20	−36	0.82	4.77	0.31	89	4.22	0.27	+29

* Calculated assuming lowest fasting output in any of other series, namely, 0.21 "doses" per 100 kgm. hrs.

The rectal temperature of the assay animals was taken at half-hour intervals after the injection of the extracts until the maximal rise was recorded. In the case of dog urine extracts we have not been able to eliminate regularly all traces of pyrogenic substances.

RESULTS. *The effects of surgical procedures.* The essential data concerning the 6 series of experiments are presented in table 1. The doses of extract administered to the assay animals are recorded in 3 ways; as milligrams and as the volume of urine, and kilogram hours represented by the milligram dose. The yields by weight of extract and of "doses" of urogas-

trone per 100 cc. of urine are included, although these do not represent a true measure of output. In measuring the urinary output of a substance, both the weight of the dog and the period of excretion must be considered. Hence, the output of urogastrone is obtained from the column headed, yield of "doses" of urogastrone per 100 kgm. hrs. Since, as will be pointed out later, the rate of urine production affects the recovery of urogastrone, the former is recorded as the volume of urine secreted per 100 kgm. hrs. The last column records the most significant figure, namely, the percentage change in urogastrone output produced by the various procedures. Since the assay dogs, the method of handling the operated animals, the extraction procedures were constant *within* any given series, but not necessarily *between* different series, it may be misleading to make any comparison other than that shown in the last column.

As shown in table 1, the removal of the entire small intestine (with or without antrectomy) reduced the output of urogastrone by 10 per cent to 80 per cent in the 4 series of experiments. A fifth series, not included in the table, showed the usual low post-operative output of urogastrone, but for an unidentified reason the extract of the fasting or pre-operative urine was completely inactive. It will be noted that in series 2 the output was reduced 65 per cent during the first two post-operative days and 80 per cent during the subsequent 3-day period.

In contrast to the above results, the output of urogastrone was increased 29 per cent and 148 per cent in the two control series in which the "dummy" operation, consisting of the exclusion of digestive secretions from the intestine, was performed.

It should be noted that following either type of operation the yields *by weight* of extract are greatly increased. This necessitated the use of large milligram doses to correspond to the pre-operative kgm.-hr. doses. The bearing of this fact on the interpretation of the results will be discussed later.

The effect of diet. The essential data concerning the 4 series of experiments are presented in table 2. In 3 series of experiments, 1 in human subjects and 2 in dogs, the feeding of a high fat diet increased the output of urogastrone 99 per cent, 23 per cent, and 194 per cent over the fasting output. However, the ingestion of a practically fat-free diet similarly increased the output 86 per cent in dogs.

The effect of peptic ulcer. Table 2 also includes the results obtained with the extracts prepared from the urine of ulcer patients. The urogastrone output was found to be 0.037 dose per 100 kgm. hrs. in these patients receiving a milk diet in contrast to the higher outputs of 0.077 and 0.153 dose respectively for normal subjects, fasting, or receiving a cream diet. The increased urine volume of the ulcer patients yielded more extract, but its potency was so reduced as to diminish significantly the output of urogastrone activity.

The effect of urine production. In the course of the above experiments it was noted that the rate of urine production influenced the apparent fasting output of urogastrone in dogs. In table 3 the yields of urogastrone "doses" per 100 kgm. hrs. and also the yields per 100 cc. of urine are presented together with the volume of urine excreted per 100 kgm. hrs. It

TABLE 2

Urogastrone outputs in fasted and fed subjects, and ulcer patients

SERIES	NUMBER OF DOGS	CONDITION	ASSAY							YIELDS					
				Dose			Effect			Per 100 cc. urine		Per 100 kgm. hrs.			
			Number of assays	Mgm.	Cc. urine	Kgm.-hrs.	Temp. rise	% inhib.	Inhib. doses	Extr.	"Doses"	Urine	Extr.	"Doses"	% change
							°F.			mgm.		cc.	mgm.		
1	4	Fasted + fluid	6	6	396	206	2.7	−47	1.18	1.51	0.298	191	2.89	0.57	
		High-fat diet	11	4	220	162	1.7	−46	1.14	1.82	0.518	135	2.46	0.70	+23
2	4	Fasted + fluid	12	5	393	205	1.3	−32	0.70	1.28	0.178	191	2.43	0.34	
		High-fat diet	11	5	235	128	1.1	−50	1.28	2.19	0.545	178	3.91	1.00	+194
3	4	Fasted	8	3	378	579	0.9	−57	1.62	0.80	0.428	66	0.518	0.28	
		Low-fat diet	9	3	478	321	0.9	−58	1.68	0.63	0.352	149	0.935	0.52	+86
	MEN														
4	28	Fasted	15	5	1235	2185	0.7	−58	1.68	0.41	0.136	56	0.229	0.077	
		Cream diet	12	5	1130	1565	0.2	−69	2.40	0.44	0.213	71	0.319	0.153	+99
5	8	Ulcer patients, milk diet	15	10	2196	1900	0.6	−32	0.70	0.46	0.032	115	0.526	0.037	

TABLE 3

Effect of urine flow on extract yields

URINE FLOW PER 100 KGM. HRS.	CRUDE EXTRACT		INHIBITORY ACTIVITY	
	Per 100 cc.	Per 100 kgm. hrs.	Per 100 cc.	Per 100 kgm. hrs.
cc.	*mgm.*	*mgm.*	*"doses"*	*"doses"*
44	40.3	17.8	0.50	0.21
66	28.1	18.4	0.43	0.28
77	40.2	30.9	0.39	0.29
191	28.5	54.5	0.24	0.45
360	20.3	73.0	0.22	0.72

can be seen that as the rate of urine excretion increases, the output of urogastrone also increases, but not at the same rate, since the concentration of urogastrone in the dilute urine is reduced. Similar relationships are obtained if the weight of crude extract is substituted for "doses" of urogastrone. It should be recalled that the curves are influenced to an unknown

extent by the variations in the procedures employed in the different fasting series.

It has been found that the degree of recovery of solid material from urine by the benzoic acid procedure may be in part responsible for the above results. For example, one liter of pooled human urine yielded 60 mgm. of crude concentrate; another liter from the same pooled specimen when diluted with an equal quantity of water and then extracted as two liters of urine, yielded 75 mgm. of crude concentrate. Hence with a constant total quantity of solids, the more dilute it is the greater the total recovery, but the less is the yield per unit volume. Accordingly, the non-quantitative nature of the extraction procedure may be responsible for the apparent influence of diuresis on the output of urogastrone.

If tables 1 and 2 are re-examined in the light of the above findings, it will be seen that variations in the rate of urine production do not account for the effect of surgical procedures or of diet on the output of urogastrone.

Discussion. If the results obtained are to be accepted at face value, they indicate that the output of urogastrone is reduced by removal of the small intestine, and increased by the induction of diuresis, by the ingestion of a high or low fat diet, and by the exclusion of digestive secretions from the small intestine. However, evidence was obtained which suggested that the apparent effect of diuresis may be due to an improved recovery of urogastrone from the urine rather than to an increased output. Furthermore, it must be remembered that the methods of extraction are not strictly quantitative, and that neither the assay nor extraction procedures are specific for a single biologically active compound. Hence, one must be cautious in the interpretation of the results.

The extent to which these deficiencies in methods may influence the final interpretation of the results is considerable. For example, more material was extracted from the post-operative than from the preoperative urines, which necessitated the use of larger doses for assay. Since the extraction procedure is not quantitative, this greater yield could be indicative of a more complete recovery of urogastrone from the post-operative urines. If this were actually the case, the effect of enterectomy should properly be revealed by a comparison of the outputs after enterectomy with the outputs after the control operation; this would imply a marked reduction in urogastrone output following removal of the small intestine. On the other hand, since the methods are not specific, it could be maintained that the extra material extracted from the post-operative urines, which probably consists of products of autolysis of the surgically traumatized tissue (8) might produce a "non-specific" inhibition. If this were actually the case, the *extra* activity after the control operation and the *entire* activity after enterectomy could be attributed to these "non-specific" inhibitor substances; this would imply that enterectomy completely eliminates urogastrone from the urine.

Although the above interpretations cannot summarily be dismissed from consideration, neither can they be accepted to the exclusion of other interpretations. In short, the results do not permit a final decision regarding the effect of enterectomy on the excretion of urogastrone. What can be said with a reasonable degree of certainty is that *a*, the exclusion of digestive juices from the small intestine (control operation) does not decrease the output of inhibitor substance or substances, and *b*, if there is only one inhibitor substance in the urine extracts, it does not originate entirely from the small intestine.

It is conceivable that enterogastrone is liberated in small quantities under fasting conditions and in larger quantities during the digestion of a low-fat meal, although there is no direct evidence bearing on these points (7). Accordingly, in these respects the behavior of urogastrone is not incompatible with the view that it represents excreted enterogastrone. However, the ingestion of fat should be the most effective stimulus for the release of enterogastrone and in this respect urogastrone appears not to behave like enterogastrone. This evidence, in conjunction with the uncertain results of the enterectomy experiments, makes the weight of evidence favor the view that urogastrone and enterogastrone are separate entities. On the other hand, the fact that enterectomy reduces, feeding augments, and the presence of a duodenal ulcer reduces the output of urogastrone, points to the importance of the gastro-intestinal tract for the control of urogastrone excretion.

CONCLUSIONS

1. The apparent excretion of urogastrone is decreased by removal of the small intestine and increased by the induction of diuresis, by the ingestion of a high or low fat diet, and by the exclusion of digestive secretions from the small intestine (control operation).

2. Since the apparent excretion may not truly represent the actual excretion, for reasons discussed, the acceptable conclusions are limited to the following:

a. The exclusion of digestive secretions from the small intestine (control operation) does not reduce the output of inhibitor substance(s).

b. If only one inhibitor substance is present in the urine extracts, it does not originate entirely from the small intestine.

c. A high-fat diet is no more effective than a low-fat diet in augmenting the output of inhibitor substance(s).

3. The output of urogastrone is reduced below normal in patients with peptic ulcer.

4. The evidence suggests the importance of the gastro-intestinal tract for the regulation of urogastrone excretion, but does not support the view that urogastrone and enterogastrone are identical.

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THYROID ACTIVITY AFTER IODINE INGESTION

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In hyperthyroid patients iodine is commonly given to depress the activity of the thyroid in preparation for an operation. In animals iodine has been shown to diminish the responses of the thyroid which are ordinarily produced by giving anterior pituitary extracts (see Siebert and Linton, 1935; Friedgood, 1936; Cutting and Robson, 1939). In order to learn more about this effect, we have studied rats during prolonged exposure to cold. The elevation of metabolism in this case is due to the release of extra hormone from the thyroid (see Ring, 1939). The increase occurs in the absence of the cervical sympathetics and is probably brought about by the anterior pituitary (Ring, 1939; Uotila, 1939). Our problem was to find out whether the metabolic response to cold could be depressed by the ingestion of iodine. First, we decided to see whether iodine affected the normal basal metabolism of rats. Only when rats were given doses of iodine so large that they lost weight, did the metabolism fall. The withdrawal of this extra iodine, however, brought about a much more decided reduction in metabolism which lasted for several weeks. We have followed this change in some detail.

METHOD. In the first group of experiments, 20 mgm. of NaI were placed in the water which each rat was expected to drink during one day (ordinarily 25 cc.). This amount was supplied daily for one week and the basal metabolism was then measured by means of a modification of Benedict's Multiple Chamber Respiration Apparatus (see Ring, 1940). Each subsequent week, the NaI added to the drinking water was doubled until the animals started to lose weight. Then water without NaI was supplied and metabolic measurements were continued at weekly intervals until they approached normal.

A second procedure consisted in giving rats drinking water which contained 0.75 mgm. of NaI per cc. Measurements of basal metabolism were made at weekly intervals for 3 weeks. These rats weighing about 200 grams each ingested 20 to 25 mgm. NaI per day. This did not affect their basal metabolism nor stop their growth. These and similar rats were then divided into 5 groups. Group A was placed in the refrigerator at 2° to 4°C. for 3 weeks and supplied with drinking water containing NaI. Group B

was given ordinary drinking water while in the refrigerator. All of the animals in group B died before measurements of metabolism were made. Therefore, in group C, the NaI was discontinued for five days before as well as during their stay in the refrigerator. Most of this group survived. Groups D and E, after the withdrawal of NaI were given 25 guinea pig units of anterior pituitary thyrotropic principle[1] per 100 grams of body weight each day for four days. Group D was then placed in the refrigerator while group E was kept at room temperature. This last group was used to study the effects of extract alone upon metabolism. Finally some normal rats were placed in the refrigerator as controls. All rats were fed on Purina Dog Chow which has been shown to contain adequate amounts of I (see Remington and Remington, 1938). This food supplied about 4 gamma of I per day when the rats were kept at room temperature and about twice that amount while they were living in the refrigerator.

Results. Table 1 shows the basal metabolism of rats during and after the ingestion of NaI in amounts large enough to stop growth. The quantity which each rat would tolerate was quite variable. The substitution of water for NaI solution permitted growth to begin again but the metabolism fell and remained subnormal for weeks. When one large dose of NaI (1200 mgm.) was given, the results were similar. The control measurements on six of these rats averaged 780 cal. per sq. m. per day. After ingesting NaI, the metabolism, measured at weekly intervals, averaged 705, 718, 724, 743. Of course the giving of such large amounts of NaI leads to a temporary loss of weight, and this in itself might lower metabolism. However when inanition is produced by fasting, the metabolism returns to normal a few days after food is supplied. It is hardly likely therefore that this would account for the prolonged changes observed.

When smaller doses of NaI (20–25 mgm. daily) were given for a period of three weeks, growth continued and basal metabolism was probably not depressed. The results are shown in table 2. The fall of 2 per cent in metabolism is due, we believe, to the animals becoming more accustomed to the apparatus. As large a drop commonly occurs in untreated animals. After stopping the ingestion of this amount of NaI, other experiments have shown that there is no fall in basal metabolism. Nevertheless, the usual responses of the thyroid to stimulation could not be evoked. This was shown in two ways—by injecting Antruitrin T and by subjecting the animals to cold over a prolonged period of time. The third table indicates the changes produced by injecting thyrotropic principle. In the control rats, these injections caused an increase in metabolism reaching its maximum of 10 per cent two weeks after the injections were started. Rats that had previously received NaI did not show any metabolic change.

[1] The thyrotropic principle, antuitrin T, was kindly supplied by Parke, Davis and Company.

Of twelve rats placed in the refrigerator at the time NaI was withdrawn, none survived for more than two weeks. Apparently the thyroid could not make an appropriate response to the cold environment so that the body temperature fell and the animals died. When the NaI was withdrawn 5 days before placing the animals in the refrigerator, 7 out of 8 rats survived but their metabolic response to cold was smaller than in normal animals, as

TABLE 1

Prolonged feeding with NaI in drinking water

RAT NUMBER	CONTROL METABOLISM WITH NaI INGESTION (CAL./SQ.M./DAY)			AFTER WITHDRAWAL OF NaI							AMOUNT OF NaI GIVEN PER DAY
	1st wk.	2nd wk.	3rd wk.	4th wk.	5th wk.	6th wk.	7th wk.	8th wk.	9th wk.	10th wk.	
											mgm.
1	768	736	696	668	652	660	692				80
1	692	687	676	627	636	669	692	703			320
2	739	740	769	662	637	754	671				180
3	722	668	677	611	589	655	680				160
3	686	670		637	643	563	610	612	657	699	40
4	734	787	774	701	693	724	691	731			180
5	772	788	675	677	713	695	717	783			180
Average met.	730	725	705	655	652	674	679				
Average wt.	278	276	262	271	275	282	290				

TABLE 2

Metabolism before and during the ingestion of NaI*

	CONTROL PERIOD		GIVEN (MGM. NaI PER CC. DRINKING WATER)	DURING NaI INGESTION		
	1st wk.	2nd wk.		3rd wk.	4th wk.	5th wk.
			mgm.			
Average metabolism of 7 rats	858 ±11.0	836	0.75	840	827	817 ±12
Average weight	224.0	230.6		238.6	246.3	251.8

* In calories per square meter per day.

shown in table 4. The difference between the two groups of animals is not large but statistically there are 39 chances in 40 that this difference is significant.

Rats given NaI during their stay in the refrigerator showed an elevation in metabolism which was at least as great as that found in the controls (see table 4). It appears that when NaI is given after the rats leave the refrigerator the metabolism returns to normal more quickly. Of the control

group 7 out of 9 still showed an elevated metabolism four weeks after leaving the refrigerator whereas none of those receiving NaI showed any elevation at this time. In fact the metabolism of the latter group was slightly depressed.

If animals from which NaI has been withdrawn are given Antuitrin T and then placed in the refrigerator they show the largest elevation in metab-

TABLE 3

Metabolism of rats after receiving thyrotropic principle*

(25 guinea pig units per 100 grams per day for 4 days)

	CONTROL		1ST WK.	2ND WK.	3RD WK.	4TH WK.
5 control rats	787	Thyrotropic	799	862	826	
6 rats after NaI ingestion	799	principle	772	801	787	773

* In calories per square meter per day.

TABLE 4

Change in basal metabolism after three weeks' exposure to cold (in per cent)*

CONTROLS	RATS GIVEN NaI UNTIL 5 DAYS BEFORE EXPOSURE TO COLD	RATS GIVEN NaI DURING EXPOSURE TO COLD	RATS GIVEN ANTUITRIN T AND NaI BEFORE EXPOSURE TO COLD
(Measured after being at room temperature for one day)			
+3.9	−1.5	+4.0	0
4.6	+1.0	7.1	+11.9
5.4	2.7	9.7	14.7
8.0	5.8	13.4	15.1
9.0	7.2	16.1	22.8
9.6	7.8	19.8	24.6
10.7	12.2		
13.5			
17.0			
Average.... 9.1 ±0.9	5.0 ±0.7	11.7 ±1.5	+14.9 ±2.2
(Measured after being at room temperature for one week)			
Average.... 4.6	+3.1	+7.0	+8.0

*Measured at 30°C.

olism of any group. This is in spite of the fact that without the cold stimulus, thyrotropic principle will not elevate the metabolism of otherwise similarly treated animals.

DISCUSSION. Our results clearly show that NaI will not prevent the usual elevation in metabolism produced by cold. According to Starr and Roskelley, the injection of NaI into rats kept in a refrigerator limits the

hypertrophy of the thyroid. It is therefore apparent that no correlation exists between the metabolic response and the histological picture under these circumstances.

When the stimulus due to the cold environment is removed, the metabolism returns to normal more quickly if the ingestion of NaI is continued. This response is somewhat similar to that observed when hyperthyroid patients are given I.

It is surprising that after giving large amounts of NaI, the withdrawal depresses thyroid function. Possibly the thyroid gland becomes accustomed to a certain level of I in the blood and when this level falls, the gland fails for a time to take up the iodine it needs to manufacture new hormone.

When rats stop taking NaI, thyrotropic principle will apparently prepare the thyroid to make a suitable adjustment to cold. In fact the response obtained was greater than either stimulus alone would produce. In two rats which did not receive NaI, thyrotropic principle seemed to increase the metabolic response to cold in a similar manner.

CONCLUSIONS

1. When rats, kept in a refrigerator for 3 weeks, are given 20 to 25 mgm. NaI per day, an elevation in basal metabolism occurs which is as great as that found in control rats (see table 4).

2. After being placed in a warm environment the metabolism of these rats returns to the control level more quickly if they are given NaI.

3. The ingestion of NaI does not depress the metabolism of normal rats unless the amount given is large enough to cause inanition.

4. The withdrawal of large doses of NaI (in most cases 160 mgm. per day) frequently depresses basal metabolism for several weeks thereafter (see table 1).

5. The withdrawal of smaller doses of NaI (20–25 mgm. per day) does not bring about a measurable reduction in metabolism. It does prevent the occurrence of as large an elevation of metabolism as that ordinarily produced by either thyrotropic principle or prolonged exposure to cold.

6. When thyrotropic principle is given and followed by prolonged exposure to cold, the metabolic response is greater than that produced by either stimulus alone (see tables 3 and 4).

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THE EFFECT OF VARYING RESISTANCE-LOAD AND INPUT-LOAD ON THE ENERGETICS OF THE SURVIVING MAMMALIAN HEART[1]

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The law of the heart enunciated by Starling (1) that the output of the heart is a function of its diastolic size, has been amplified to apply also to its work and energy expenditure by Starling and Visscher (2). This would indicate that the relation: work/energy expenditure, i.e., the mechanical efficiency of the heart, is also a function of the diastolic heart size. However Gollwitzer-Meier et al. (3, 4) and Gremels (5) have indicated that the mechanical efficiency of the heart is also dependent upon whether the change in size is induced by a change in the arterial resistance or by a change in the venous return. They reported that the augmentation of the work of the heart noted on increasing the arterial resistance is accompanied by a much greater increase in energy expenditure than a like augmentation of work observed on increasing the venous return to the heart. The mechanical efficiency at a given increased level of work would thus appear to be less when the work increase is due to an increased resistance-load than when it is due to an increased input-load.

Objections can be raised to the methods used by these investigators. Gollwitzer-Meier et al. (3, 4) assumed that coronary sinus blood was a representative sample of the mixed coronary venous blood as far as O_2 content is concerned, and this has been shown to be not necessarily true (6). Gremels (5) assumed that the coronary sinus flow was always 60 per cent of the total coronary flow, and this too has been shown to be erroneous (7, 8).

It was deemed advisable, therefore, to reinvestigate the subject employing a method which would measure more accurately both the total work of the heart and its total energy expenditure based upon oxygen consumption while doing this work.

In this study we utilized either the isolated heart preparation which we have described previously (9, 10) or a special heart-lung arrangement in which the circulating blood volume could be adjusted (fig. 1). The input-

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load was altered in the former by changing the resistance to inflow from the blood reservoir, in the latter by changing the circulating blood volume. The resistance-load was altered in both preparations by adjusting the resistance in the left and right heart outflow circuits. In both preparations, when the rate of inflow was changed, the peripheral resistances were adjusted so that the systemic and pulmonary arterial pressures (the resistance-load) were kept constant. Likewise when the resistance-load was changed, the inflow or the circulating blood volume was adjusted so that the cardiac minute output (the input-load) was kept constant.

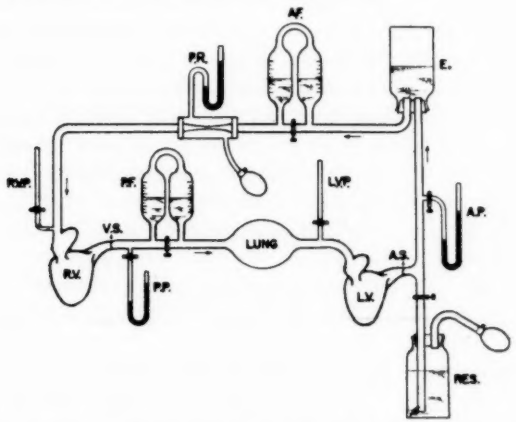

Fig. 1. Diagram of the closed-circuit heart-lung preparation. *L.V.P.* is the left venous pressure manometer; *L.V.*, the left ventricle; *A.S.*, the point where the aortic blood sample is taken; *RES*, the blood reservoir which is used to add or remove blood from the heart-lung circuit, this is controlled by the screw clamp on the tube connecting it to the heart-lung circuit and by the pressure bulb; *A.P.*, the aortic pressure manometer; *E.*, the artificial arterial elastic reservoir; *A.F.*, the aortic flowmeter; *P.R.*, the manometer measuring the degree of artificial peripheral resistance which is controlled by a pressure bottle and bulb; *R.V.P.*, the right venous pressure manometer; *R.V.*, the right ventricle; *V.S.*, the point where the pulmonary blood sample is taken; *P.P.*; the pulmonary arterial pressure manometer; and *P.F.*, the pulmonary artery flowmeter.

The work of the two ventricles was measured as in our previous studies (9, 10). Energy cost was computed as before, but the method of measuring O_2 consumption was altered to avoid the criticism applied to our previous work that the coronary sinus sample was not a true mixed venous sample. The blood samples for O_2 determination in this study were drawn under oil simultaneously from the aortic and pulmonary arterial tubes, the former giving the arterial blood, the latter the mixed venous blood. The actual O_2 content measurements were carried out as before (9) by the Van Slyke method and the A-V O_2 difference determined from the difference in O_2

content of the two samples. This multiplied by the total flow in the pulmonary artery at the time the samples were taken gave the O_2 consumption of the heart, viz:

$$O_2 \text{ consumption (cc./min.)} = \frac{\text{A-V } O_2 \text{ difference (vol. per cent)} \times \text{pulmonary arterial flow (cc./min.)}}{100}$$

This was possible because the blood in the pulmonary artery is a mixture of (1) blood that has passed through the heart cavities without the loss of oxygen, i.e., aortic blood, and (2) blood that has passed through the coronary system and has had oxygen removed from it by the heart muscle. The only cause for the difference in oxygen content between aortic and pulmonary samples is thus the O_2 utilization of the heart[3].

If P_f, A_f and C_f represent in cc./min. the flow in the pulmonary artery, aorta and coronary system respectively, and P_o, A_o and C_o represent the O_2 content of the blood in vol./100 cc. in the pulmonary artery, aorta and the true mixed coronary venous blood, respectively, then

$$P_f P_o = A_f A_o + C_f C_o$$

from which

$$C_0 = \frac{P_f P_0 - A_f A_0}{C_f}.$$

Since O_2 consumption $= C_f (A_o - C_o)$, it follows that

$$O_2 \text{ consumption} = C_f \left\{ A_0 - \frac{(P_f P_0 - A_f A_0)}{C_f} \right\}, \text{ or}$$

$$O_2 \text{ consumption} = C_f A_o - P_f P_o + A_f A_o, \text{ or}$$

$$O_2 \text{ consumption} = (C_f + A_f) A_o - P_f P_o.$$

Since $P_f = C_f + A_f$,

$$O_2 \text{ consumption} = P_f (A_o - P_o),$$

that is, the O_2 consumption of the heart can be accurately measured by the product of pulmonary flow and A-V O_2 difference.

Four preparations were used, 3 special heart-lung preparations and 1 isolated heart preparation. Ether was the anesthetic agent employed until the blood supply to the head was interrupted. The aeration from this point until the preparation was ready for use, a half-hour at least, was sufficient to remove most, if not all, of the ether from the blood. Heparin was used as the anticoagulant in the dog from which the preparation was made and also was added to the defibrinated blood obtained for circulation. Details of preparation were carried out as previously, the only variation being

[3] The coronary venous return via Thebesians to the left heart, which is small and almost constant, estimated as 6 ± 4 per cent of the coronary flow (8), is the remaining source of error.

in the special heart-lung preparation. Here the lungs were left in situ and a blood reservoir was connected to the aortic circuit via a side arm which was kept clamped except when changes in blood volume were desired (fig. 1).

When the preparation was ready, the dynamic conditions set, and the heart stabilized to them, control blood samples, pressure, flow, peripheral resistance, heart rate and blood temperature readings were taken. The readings were repeated every 1 to 2 minutes. Two or three control blood samples were obtained at approximately 15 minute intervals. The work level was then raised by increasing either the resistance- or the input-load, the other being kept constant. Usually two more sets of blood samples were taken and then the work restored to its previous level by reducing the factor which had been increased. Another set of blood samples was taken at the reëstablished control level and the work then increased to about the same level as before, but this time by increasing the load which had previously been kept constant, the other load now being unaltered. Again, after a period of observation during which two sets of blood samples were taken, the work was reduced to the control level and a final set of blood samples taken. From these data the work, O_2 consumption and mechanical efficiency could be calculated as in our previous studies, differing only in that the new method of determining O_2 consumption, as described above, was employed.

Results. The results are summarized in table 1, and illustrative experiments are shown in figures 2 and 3.

Control values. The control values in these four experiments ranged as follows:

Aortic pressure	86–101 mm. Hg
Pulmonary arterial pressure	16–27 mm. Hg
Pulmonary artery flow	98–175 cc./min.
O_2 consumption	2.7–4.0 cc./min.
Work	9–17 kgm.M/hour
Efficiency	2.2–4.3 per cent

Effect of increasing the resistance-load. As the results in table 1 show, the increase in arterial blood pressure was accompanied by a slight but consistent decrease in the average values of mechanical efficiency, that is, the O_2 consumption increased out of proportion to the increase in work. This enhancement of O_2 consumption was greater in the 1st determination after the increase in work than in the 2nd one. At the time of the 2nd determinations the efficiencies were well within the control range. We do not consider the slight average decrease in efficiency to be of significant degree although it was consistent. *There was no instance of any increase in efficiency.* During the period of increased resistance-load the coronary flow rose from 8 to 75 per cent above the control level. In two of the heart-lung

preparations the pressure in the pulmonary veins rose also. In all instances the heart increased in diastolic size on inspection.

Effect of increasing the input-load. As the results in table 1 show, the increase in venous inflow was accompanied by a definite and consistent increase in the average values of mechanical efficiency, that is the O_2 consumption did not increase in proportion to the work. In fact, in the isolated heart preparation, the 2nd determination showed an O_2 consumption lower than in the control. In the other preparations, the O_2 consumption rose somewhat, and at the time the 2nd samples were taken had returned to within the control levels.

TABLE 1

Effect of increased work on mechanical efficiency of heart

PREPARATIONS	INCREASE IN RESISTANCE LOAD					INCREASE IN INPUT-LOAD				
	Time of readings after dynamics were altered	Increase in work	Average control efficiency	Average efficiency during work increase	Change in efficiency with increase in work	Time of readings after dynamics were altered	Increase in work	Average control efficiency	Average efficiency during work increase	Change in efficiency with increase in work
	min.	*per cent*	*per cent*	*per cent*	*per cent*	*min.*	*per cent*	*per cent*	*per cent*	*per cent*
Heart-lung 1	13 and 19	50	3.2	2.6	−20	5 and 11	100	3.2	5.3	+65
						10 and 20	50	3.1	3.8	+20
Heart-lung 2	15	30	4.3	3.4	−25	6 and 13	50	3.7	7.0	+90
Heart-lung 3	9 and 14	30	3.5	3.1	−10	11 and 22	25	3.5	4.9	+40
Isolated heart	15 and 25	22	2.3	1.9	−15	18 and 24	26	2.4	3.9	+60
Range of efficiency in per cent on increasing work					−10 to −25					+20 to +90

The coronary flow did not alter appreciably except in the isolated heart preparation, where it increased during the period of increased work. The right venous pressure rose in two of the heart-lung preparations. In all instances the heart increased in diastolic size on inspection.

Discussion. Our results are thus in general agreement with those of Gollwitzer-Meier and Gremels, and the objection raised to the accuracy of their work is overcome. Increasing the load of the heart increases its work, but the degree to which its oxygen consumption is increased depends also upon the nature of the increased load. Within the range employed in these experiments increasing the resistance to emptying of the heart leads to an increase in oxygen consumption in proportion to or greater than the increase in work; therefore, no increase in the mechanical efficiency of the heart occurs and, at times, a slight decrease actually appears. Similarly increasing the minute volume output of the heart within the limits studied

leads to a definitely improved mechanical efficiency; in fact, at times the O_2 consumption does not appear to increase at all.

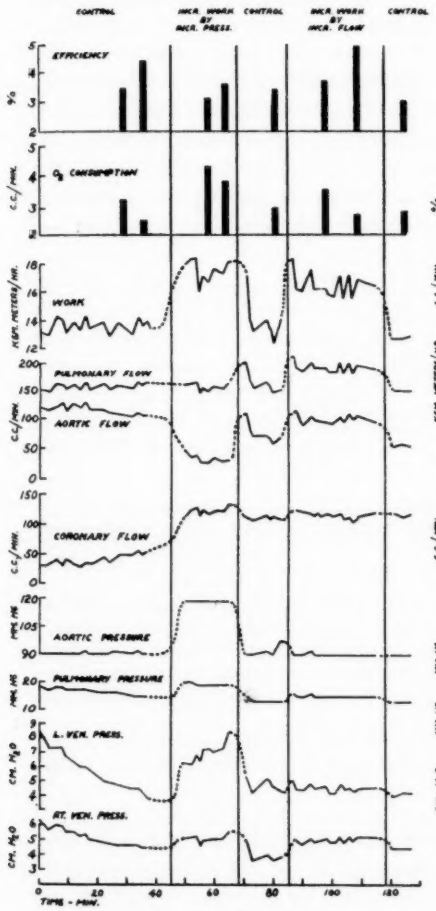

Fig. 2

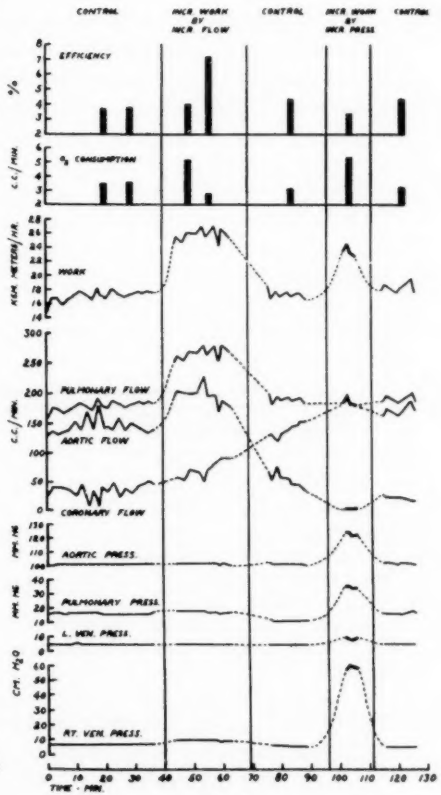

Fig. 3

Fig. 2. Chart listing pertinent data on one (heart-lung preparation) of four experiments discussed in text. *L. ven. pressure* and *Rt. ven. pressure* are left and right venous pressures. *Incr.* equals increased. Dotted lines are used to connect periods where no observations were made.

Fig. 3. Chart listing pertinent data on another experiment (heart-lung preparation) discussed in text. Conventions as in figure 3.

These results therefore cannot be taken to confirm the concept in the surviving heart that the increase in heart size which is associated with increase

in load is always accompanied by an increase in mechanical efficiency as has been assumed in the past. Both methods of increasing the work of the heart, namely, by increase of resistance-load and by an increase of input-load, are accompanied by an increase in heart size clearly seen on inspection and yet the results as far as mechanical efficiency is concerned are different. This can be shown by plotting work against O_2 consumption. Two such graphs are shown in figures 4 and 5. In each it will be seen that while increase in work is accompanied by a tendency for the O_2 consumption to increase, the increase in the latter is much greater when the resistance-load is responsible for the increased work than when this is due to an increased input-load. The lines connecting the control values with those of the in-

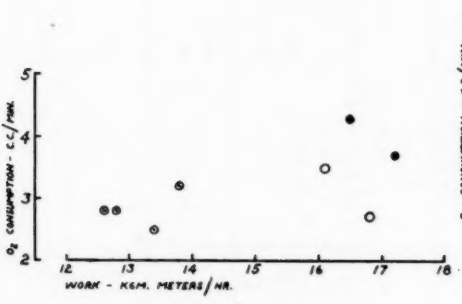

Fig. 4

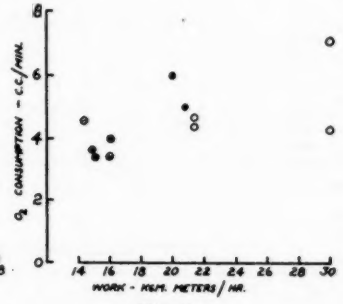

Fig. 5

Fig. 4. Chart correlating work and O_2 consumption in the experiment shown in figure 2. ⊙, are control observations; ○, observations after increase only in input-load; ●, observations after increase only in resistance-load. Note that the O_2 values for the input-load increase are lower than those for the resistance-load increase. In addition, the second observation with each of the increased loads is lower than the first. Discussed in text.

Fig. 5. Chart correlating work and O_2 consumption in another experiment. Conventions as in figure 4. The changes noted in figure 4 are shown here also. Discussed in text.

creased work under the two conditions do not coincide as would be demanded if heart size were the only factor involved. It follows therefore that other factors in addition to heart size determine the efficacy with which the heart utilizes its energy for its work.

The possible objection that the difference between these two types of increased load is due to an increase in the kinetic energy factor is not valid since under the conditions of the experiments the latter is of the order of $\frac{1}{500}$ of the potential energy used to calculate the work.[4] It is apparent then

[4] For example, in figure 3 at the time $t = 54$ minutes, where the flow is greatest and the kinetic energy should be at a maximum, the kinetic energy can be computed as follows:

that not only the size of the heart but the magnitude of the after-load, which is what the resistance to emptying really is, modifies the ability of the heart to utilize its energy for work.

Starling's law may thus be more precisely considered. While increasing heart size tends to increase heart work (1), and oxygen consumption (2), the increase in efficiency which is stated to occur (2) holds only when the after-load, the resistance to emptying, is not altered. Increasing the latter tends to reduce the mechanical efficiency and in our experiments this tendency was sufficient to nullify and sometimes even to overbalance the effect of increase in heart size tending toward increase in efficiency. It is obvious then that to define work, oxygen consumption and efficiency, it is necessary to consider the nature of the load as regards initial load (venous return), and after load (resistance to emptying), as well as in regard to heart size.

Clinically these facts are of significance since in disease, hypertension of the systemic and/or pulmonary circuits occurs with and without changes in cardiac output. Also, changes in cardiac output may occur with no change or an insignificant change in the after-load. The energy expenditure per unit of work would be different under these circumstances due to more than the change in heart size, assuming the same factors operate in man.

Our results permitted us also to demonstrate again the absence of a significant oxygen debt after the periods of increased work. Any oxygen debt is so small and fleeting as not to significantly alter the O_2 consumption after the heart had been restored to its preëxisting work level. This is in accord with the concept of Katz and Long (11) and confirms the observations on this point of Gollwitzer-Meier (3).

An interesting observation revealed by our experiments is the tendency of the oxygen consumption at the time of the 2nd sample to be less than at the time of the first sample during both periods where the work was increased. As a result there was an increase in efficiency as the increased work was maintained. This would imply that there is an adaptation to the increase in work on the part of the heart. In the instances of increase in initial load, the later O_2 consumption came to lie within the control values in some of the experiments. The nature of this adjustment is problematic.

It is possible that these factors of initial load and after-load operate also in the failing heart and failure to recognize this has perhaps been the cause,

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$K_E = \frac{Mv^2}{2g}$ gm. cm./sec., where M = mass of blood put out by heart per second; v = linear velocity of blood leaving heart in cm./sec.; g = 980 cm. sec.^{2}.

Where M = 2 times the pulmonary flow/sec. = 9.2 grams, and the diameter of the pulmonary artery and the aorta together is 0.6 cm. or more, K_E max. = 0.046/kgm. meters/hr. and $\frac{K_E \text{ max.}}{\text{work as calculated}} = \frac{0.046}{27} = \frac{1}{500}$ approximately.

among other things, of discrepancy in the results of studies on the failing heart. It is conceivable that in the failing heart even when these loads are unchanged the decrease in the ability of the heart to perform its work may make them relatively increased and hence under these circumstances they may come to represent the equivalent of an increase in load.

SUMMARY

A method is described for obtaining a true mixed coronary venous blood sample in the heart-lung or isolated heart preparation of the dog, and for calculating the true oxygen consumption of the heart.

Measurements of oxygen consumption and work of the heart preparation indicate that the increase in oxygen consumption consequent upon an increase in work depends not only upon the magnitude of the work increase, but also on whether the augmented work is produced by raising (1) the venous return or (2) the peripheral resistance. In the former case the increase in oxygen consumption is proportionally less than in the latter, so that a given amount of work is done more efficiently with a large venous return and a low peripheral resistance than the same amount of work done with a low venous return and a high peripheral resistance.

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THERMAL SENSATION AND DISCRIMINATION IN RELATION TO INTENSITY OF STIMULUS

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The object of this report is to present new data on the relationship of the intensity of a thermal stimulus to 1, the intensity of the sensory response, and 2, the discriminatory ability for temperature sensation.

The present work employs a modification of the radiation techniques formerly used in this laboratory (1) which lends itself especially well to this type of problem. The methods to be described use exclusively radiation as the stimulating agent, a procedure to which decided advantages accrue. It avoids any actual material contact between subject and stimulator, obviating thereby the arousal of unwanted sensations such as touch. This allows the subject to experience the desired thermal sensation undistracted by other factors. In addition, there are no mechanical effects resulting from this type of stimulus so that there is no interference with blood flow nor any distortion of the skin introduced as experimental artifact, whereas methods employing actual material contact of the subject with a stimulator cannot be wholly free from such effects. Besides having these advantages, the method is flexible and the stimuli and limens are easily made quantitative in the same absolute units. The Weber ratio thus obtained is therefore more significant than when it is obtained from measurements expressed in degrees Centigrade or other arbitrary systems.

Many studies of thermal discrimination and of the Weber-Fechner law have been made in the past, the net result of which has been to establish the Weber-Fechner law

$$S = k \log I + b \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

within certain limits, but to offer conflicting statements concerning the Weber law

$$\Delta I/I = C \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

In these equations, I is the intensity of the stimulus, ΔI is the least perceptible increment (that is, the least amount by which two stimuli can

differ in intensity and still be recognized as being different), S is the sensory response, while k, b and C are constants. The work of Culler (2) offers a possible clue to the reason for the confusion in data relating to the Weber law, for it leads to the conclusion that the Weber law is obeyed for absolute limens, i.e., for the smallest change in stimulus that can be perceived after the skin had been adapted to a given temperature level, but not at all for differential limens, that is, for differentiation between two stimuli acting on unadapted skin. The reference cited contains a bibliography up to 1926.

Hardy and Oppel (1) suggested that the Weber-Fechner law was obeyed for absolute limens (with the skin adapted to room temperature) within the comparatively narrow range of intensities in which they worked.

I. Sensory Response and Stimulus Intensity. Apparatus and procedure. Studies were first made with heat radiation which was periodically interrupted. The apparatus is shown in figure 1. Light from a 1,000 watt tungsten filament lamp, B, was focused by the lens, L, on the skin of the subject located directly behind the circular opening of the opaque screen, D. The size of this opening could be varied in discrete steps by the use of shields. A motor, M, through a suitable pulley arrangement, rotated the half-sector disc, S, so that each revolution of the sector gave an interval of radiation followed by an equal interval of dark. The speed of rotation could be controlled by the rheostats R and R^1. A rheostat (not shown in the figure) in series with the bulb, B, allowed the intensity of the light to be set at any desired value.

To insure that no radiation could penetrate beyond the surface of the skin, the forehead of the subject was blackened with India ink, and a central area was exposed to the radiation by placing the forehead just behind the opening of the screen, at F.

Intensity of radiation was measured by means of a radiometer which could be held at F when the forehead was removed. (In order to avoid cumbersome terminology, the radiation intensities will be reported as "units" where one "unit" is 10^{-5} gram calories per square centimeter per second.)

Previous workers (3) (4) have made use of intermittent radiation for the study of heat sensation. However, they did not measure the intensity of the radiation directly, but inferred ratios of intensity from current changes in the electrical circuit containing the heating element, and expressed their intensities in terms of the threshold intensity. Also, the importance of blackening the skin so as to localize the effect of the radiation at the surface and prevent penetration (5) does not seem to have been appreciated by these earlier experimenters.

When the forehead was in place, and the sector rotating, the exposed area gained heat during the interval of radiation and lost some of it (but not all)

during the time the radiation was cut off by the opaque half of the sector. Thus, for frequencies of rotation not too slow, the forehead would at first gain more heat than it lost, and its temperature would be fluctuating around a rising baseline. After a short time the forehead came to "equilibrium," it lost during the "dark" half of the cycle an amount of heat equal to that which it had gained during the "lighted" half so that its temperature

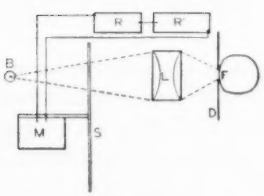

Fig. 1

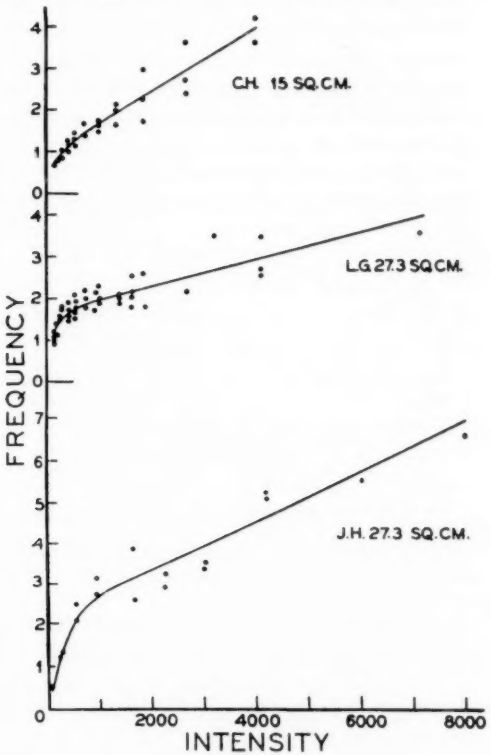

Fig. 2

Fig. 1. Diagram of the apparatus (meaning of letters explained in text).
Fig. 2. Fusion frequency (flashes per second) vs. units of intensity.

oscillated regularly along a steady baseline. It was after this steady state had set in that readings were taken.

The sensation accompanying this temperature oscillation was one of alternate pulses of warmth and cooling for the lower frequencies of rotation. As the frequency was increased, the warm pulses seemed to overlap, and the sensation became one of rippling warmth. By pushing the frequency still higher, this ripple was caused to become less and less distinct until

finally it fused into a sensation of continuous warmth. The frequency for which the ripple just disappeared was taken as the end point for the test. This end point is not a sharp one, and some practice is necessary if it is to be determined with a fair amount of certainty. For this reason the authors of this paper were the subjects and each one performed his own manipulation of the rheostat which controlled the rotation frequency. The end point for each of the tests in a run was taken a number of times and the results averaged: the extreme values of a set were rarely more than ± 6 per cent from this average. Suitable precautions were taken against returning by memory to a previous setting by changing pulley ratios, by blindly setting R^1 to an unknown value and adjusting the frequency using R alone, etc.

A. *Studies with flickering heat radiation.* Plots of the fusion frequency vs. intensity obtained in the manner described above are shown in figure 2.

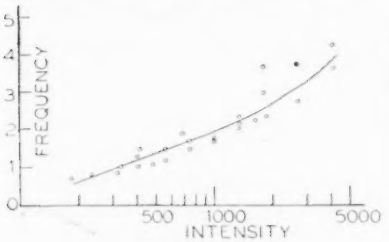

Fig. 3

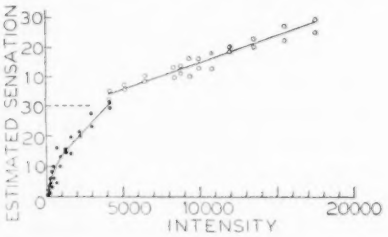

Fig. 4

Fig. 3. Fusion frequency (flashes per second) vs. units of intensity (logarithmic abscissa) for area of 15.0 sq. cm.

Fig. 4. Estimates of sensation vs. units of intensity. Data represent two separate runs (open and filled circles respectively).

The fusion frequency, after rising more or less rapidly at the lower intensities, swings into a gentler slope at roughly 1,000 units and is thereafter a linearly increasing function of intensity. The comparatively wide spread of the points is due in part to the fact that the curves are composites of runs taken on different days, and each observer was usually found to vary slightly in his level of sensitivity from day to day. One set of data (C. H.) is shown replotted against the logarithm of the intensity in figure 3. This shows a straight line up to about 1,000 units, followed by a non-linear portion. The significance of these plots will be discussed later, as they are intimately connected with the results presented in the next section.

B. *Studies employing graded estimations of sensation.* The relationship of the intensity of the sensory response to the intensity of stimulating radiation was tested in a manner similar to that recently used by Jenkins (6). A stimulus of 4,210 units was presented for two seconds to the subject and he was told to call the resulting sensation 10. He was

asked to bear this sensation in mind and to compare it with sensations which were to follow. An intensity which the subject felt to be half as strong as the standard he was to call 5, an intensity one-tenth as strong, 1, etc. He was then stimulated with intensities ranging from 83 to 4,210 units in a random manner, being always in ignorance of the intensity selected and of his progress in the test. The cycle of intensities was repeated three times, each time in a different order. Between the tests the subject was allowed to rest, and at the beginning of each set the standard intensity was given to refresh his memory.

The individual's score was added for each intensity. The results of this test are shown as the first part of the curve of figure 4 (filled circles), each circle representing the sum of the three separate estimations of sensation of one individual plotted against the intensity of the corresponding stimulus. A similar test was made separately in the range 4,260 to 17,640

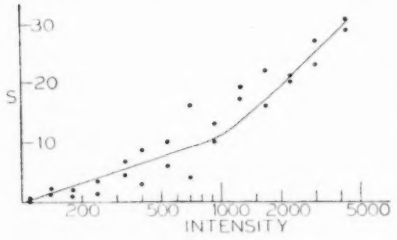

Fig. 5

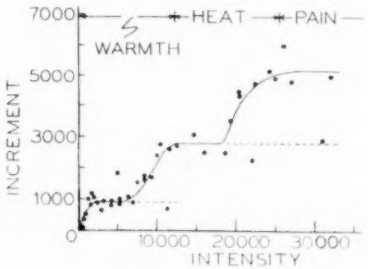

Fig. 6

Fig. 5. Estimates of sensation, S (ordinate) vs. intensity (logarithmic abscissa).
Fig. 6. Least perceptible increment (Weber increment, ΔI) in "units" vs. intensity in "units."

units, the latter being assigned the value of 10. The sensation rose linearly with the intensity as may be seen from the second part of figure 4 (open circles). This second part is obviously an extension of the first.

The similarity of the curves in figure 2 to that in figure 4 is at once apparent. Both show an initial rise up to 1,000 units followed by a linear portion whose slope is less than that for the portion of the curve below 1,000 units.

When treated in a slightly different way, the data for the preceding test will show clearly several points. Figure 5 shows the estimates of sensation as linear ordinates plotted against stimulus intensity on a logarithmic abscissa. Here again this test gives a curve remarkably similar to the corresponding treatment of the flicker data (fig. 3). Both show straight line portions up to about 1,000 units, followed by a non-linear curve. It will be seen on closer inspection that the two curves are practically superimposable point for point, if the arbitrary scale for sensation be adjusted

by multiplication by a scale-constant. The conclusion can then be drawn that the fusion frequency is truly representative of the sensory response.

The straight line plot of sensory response vs. intensity on a logarithmic scale below 1,000 units, as shown in figure 5, indicates adherence to the Weber-Fechner law. Likewise the fusion frequency data of figure 3 can be represented below 1,000 units by the Ferry-Porter law,

$$N = a \log I + d$$

where N is the fusion frequency, I the intensity, and a and d are constants. Since figures 3 and 5 show such close similarity, it is clear that the Ferry-Porter law is a variation of the Weber-Fechner law. When the results of the next section are presented it will be seen that the change in slope of these curves around 1,000 units is of physiologic significance.

II. *Thermal Discrimination.* The flicker method does not allow of direct measurement of the Weber increment (least perceptible increment), so for a direct evaluation of ΔI, recourse was had to the following method:

The rotating sector was set at a constant frequency of 0.5 cycle per second, and again a shutter was interposed so that by lifting it the operator could present a single stimulation pulse lasting two seconds. For low intensities the 15 cm.^{2} opening was used. A thin metal rod, to which was fastened a semicircular disc of copper, was mounted before the opening in such a manner that it cut off radiation from half the area, and could be flipped from side to side so that either the right half or left half of the area was darkened. A measured stimulus, I, was then presented to the subject on one side, and after a short pause a slightly greater stimulus, I_2, was presented on the other side. This test was repeated with the second stimulus being made larger than the first by small steps until the subject indicated that he could just distinguish between the two. In this way the least perceptible increment was directly obtained:

$$\Delta I = I_2 - I_1$$

For high intensities this method was modified in part to avoid stimulation of too large an area with a strong stimulus (sometimes the sensation was painful). Also, at high intensities an asymmetry in responding end organs became noticeable over the central portion of the forehead although no reliable indications of such an asymmetry could be obtained for this area at low intensities. The modification was this: The flip disc was removed, an area of 3.5 cm.^{2} used, and the two stimuli were presented over the same area with an interval of 30 to 60 seconds between them.

To insure that these two variations of the method were consistent with each other, they were used in an overlapping region (5,000 to 6,400) and were found to give identical results. The skin on which these tests were made had been adapted to room temperature, and it should be remarked

that the short duration of stimulus flash in the procedure allowed little adaptation to the radiation to occur.

Thus, of the results reported, those up to 5,000 units were obtained by the first method, those from 5,000 to 6,400 units by either of the two, and those past 6,400 units by the second method. Figure 6 shows a plot of ΔI vs. I. The curve is readily broken up into six regions. In region A, ΔI is a linear function of I, increasing as the intensity of the stimulus is increased. In region B, ΔI remains constant at a value of about 890 units. In region C, ΔI again assumes increasing values as the stimulus is increased, becoming constant in region D at a value of 2,670 units. In region E, ΔI once more resumes its upward trend with increasing intensity of stimulus and finally levels off in region F to a constant value of about 5,050 units.

To assure that there was no asymmetry introduced into the results by the method, tests were made to determine whether the decrement from a high to a lower intensity was the same as the increment from a low to a higher. There was found no difference between increment and decrement, i.e., the same number of units of intensity had to separate two stimuli if they were to be distinguishably different, no matter whether the higher were gradually decreased or the lower increased, a result which, of course, was expected.

The behavior of the Weber increment shows that the discriminatory ability of the skin is best when the discrimination is to be made at temperature levels close to the normal skin temperature, that is, for low intensities. As the intensity of the stimulus increases, the skin loses relatively more of its discriminatory powers. This loss does not proceed indefinitely, however, since the Weber increment becomes constant at stimuli between 1000 and 1500 units and presumably would remain so were the situation not complicated by the entry of other factors. For the particular circumstances of this experiment, the discrimination at the skin temperature levels is such as to allow a change in skin temperature of 0.002°C. per second to be detected after two seconds. The level of constant discrimination is attained for temperatures approximately 0.2°C. removed from skin temperature; here a change in skin temperature of 0.06°C. per second can be detected after two seconds. This degree of discrimination holds for temperatures as far removed as ±1.0°C. from skin temperature. It must be remembered that these propositions are valid only for a purely thermal stimulus such as heat radiation. Other circumstances would prevail with thermal stimuli accompanied by touch.

In regions A, B and C the sensation was one of warmth—a mild, pleasant, diffuse sensation. Just at the start of D the sensation assumed the quality of heat; this was sharper and sometimes stinging, but still diffuse and it continued through D and E. At the start of the F region the sensation became a painful one: sharp, biting, and granular. The heat and pain

thresholds were determined experimentally by presenting to the subject a series of intensities scattered randomly and asking for a report as to the quality of the sensation. The intensities at which the sensations of heat and pain first made their appearance were quite definite.

Conjecture as to the causes for the general shape of the curve may be made along the following lines. In region *A* there may well be operative some kind of statistical law governing the responses of the end organs—such a law might easily result from the nature of the matrix surrounding the end organs. It seems very likely that a group of receptors such as those in the skin, surrounded by a system of capillary pipes through which blood is coursing, near sweat glands which doubtless have intermittent periods of activity, and imbedded at different levels, should by the very nature of their position and of the inhomogeneity of their surroundings differ somewhat in their excitability; differ, that is, from each other and also show individual variation as time goes on. An interpretation along these lines is that in region *A* the warmth end organs which effectively respond to the stimulus increase in number as the intensity is raised until at its end the whole number is responding at least minimally.

That something of this nature is the case may be gathered from the work of Geblewicz (7) who measured the time required for a thermal stimulus to arouse maximum sensation and found that this time was longer, the greater the stimulus intensity. Such a result would indicate that the higher intensity is gradually bringing into activity those end organs which, probably because they lie deeper, are not so quickly excited by the stimulus and those which, because of other variable factors, have a higher threshold. Further, the change in slope of the curves obtained by the flicker method and by estimates of sensory response supports the idea that around 1,000 units or so a definite change in response occurs. In region *B* the skin is evidently detecting an absolute difference and not a fractional increase. This may be due to the fact that now all end organs are responding superminimally, although the reason why this should give a constant increment is obscure if, indeed, the mechanism proposed obtains. Region *C* denotes the entrance of a new factor (*C* factor) as is evidenced by resumption of rise of the increment. However, in this region the conscious sensation is still that of warmth and it is not until *D* is reached that sensation of heat actually is identified as such.

Possibly *C* and *D*, as well as *E* and *F*, are mechanisms analogous to *A* and *B*. In *E* and *F*, although the *E* factor enters to cause the increment to rise progressively above the flat *D* region, the sensation of pain does not become evident until *F* is reached.

Occasionally a point is obtained which lies comparatively far off the best curve which can be fitted to the data. When this deviation is towards a higher increment than is normal for that region, the explanation most

likely is that some sweating occurred, which would thus raise the increment above its true value. This explanation cannot hold, however, for some of the points which lie well below the curve. The obvious explanation for these latter is that the area stimulated, by chance, contained no active end organs of a given type; therefore, the system was thrown back to the next lowest level. To take a definite example, consider the point at 31,250 units. If during this test there were no pain receptor available for excitation, then the sensation would be one of intense heat corresponding to excitation of the warmth receptors and the *C* factor; and, indeed, for this case the point lies tolerably well on the dotted extension of the flat region proper to them. The fact that a few times a "renegade" sensation was obtained (heat, for instance, where there should have been pain) lends credence to such belief.

It might be inferred from this that the *AB*, *CD* and *EF* regions are really in a sense distinct curves superimposed one on the other, and that the break from the flat portion of one to the rising portion of the next is really a sign of the entry of a new factor into the sensory field. It is worthy to note that precisely at the bends did the subjects show most uncertainty during the tests and that the spread was greater here than elsewhere.

The trend of the curve makes evident an impairment of the discriminatory sense just before the new sensation is recognized. This is an important consideration, as it indicates that the threshold of *excitation* for both heat and pain probably lies below the actual *sensory* threshold for these sensations, an expected result. A study of this kind may objectively reveal end organ activity which will eventually be forced on the consciousness as a sensation when its intensity becomes high enough. Thus, before the subject is aware of either heat or pain sensation, sub-sensory responses of these modalities might be detected.

Although Hardy, Wolff and Goodell (8) report the pain threshold as 24,000 units, it is probable that the pain neurones are excited by 18,500 units. Similarly, the heat threshold is probably about 5000 units lower than that at which the sensation is first perceived. Further, the conclusion that the excitation threshold for pain is lower than the sensory threshold lends added support to the contention of the above authors that pain shows no spatial summation. These workers found that the pain threshold did not decrease when larger areas were stimulated but were not able to prove that the sensory threshold was not also the excitation threshold for pain. The present work indicates that the pain receptors are already aroused to sub-sensory activity before the sensory threshold is reached. Therefore, increasing the area would demand reduction of the stimulus intensity necessary to elicit threshold pain, had pain the property of spatial summation.

Figure 4 gives the subjective estimate of sensory response to the intensity

of stimulus presented, and covers the region from threshold well into the *D* portion of figure 6. Sensation seems to increase without regard to the behaviour of the Weber increment. However, it is not surprising that the *C* region does not show on figure 4, for here the increment is scarcely smaller than the extent of the region itself. The situation could not be remedied by taking a large number of points close together, for the steps would be separated by less than the necessary increment.

It appears certain that the intensity of the sensory response increases constantly as the stimulus is made progressively stronger, even in the regions where the Weber increment is constant.

That there is no conflict between figures 4 and 6 becomes apparent when it is realized that figure 6 is really the slope of the sensation curves of figure

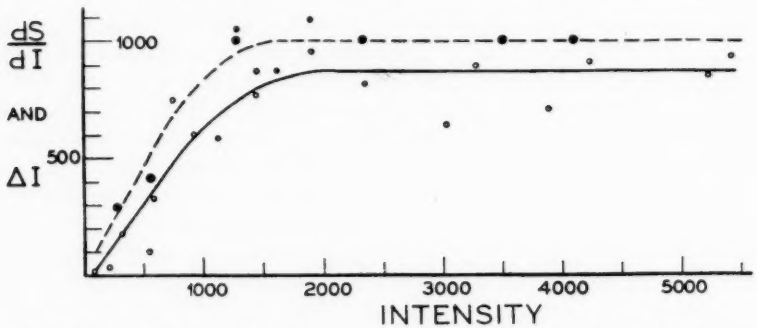

Fig. 7. dS/dI is the slope of the sensation vs. intensity curve (fig. 4) (solid circles); ΔI is the Weber increment (open circles). The former quantities were multiplied by an arbitrary constant to adjust them to fit the scale for ΔI. Both quantities are then plotted as ordinates against units of intensity as abscissae.

4. If the slope of figure 4 be taken at convenient points and plotted with the corresponding range of figure 6 as was done to obtain figure 7, the points are seen to be entirely consistent with the curve for the Weber increment. In constructing figure 7 the slopes from figure 4 were multiplied by an arbitrary constant to adjust the scale for comparison. This can be done without affecting the validity of the comparison as the scale for the sensory evaluation was purely arbitrary.

It is an interesting point to note that figure 6 allows one to determine how many discriminable steps of heat can be distinguished from threshold stimulus to a painful stimulus. If the threshold be taken as the lowest possible, i.e., the excitation threshold of the receptors, then there appear to be about 30 steps of thermal sensation distinguishable between this threshold and the onset of pain.

SUMMARY

1. The flicker method for studying temperature sensation is presented and is found to give results in harmony with results obtained by direct estimates of sensation. Wider use is made of the subjective estimates of degrees of sensation than has hitherto been generally attempted, and this method has been found thoroughly reliable.

2. The Weber-Fechner law was found to hold in a limited range (up to $1{,}000 \times 10^{-5}$ gm. cal. per sq. cm. per sec.).

3. The Ferry-Porter law is demonstrated to be a special case of the wider Weber-Fechner law.

4. A study of the Weber increment shows that it may either change with the intensity, or be a constant, depending on the range. The behavior of the increment is such as to suggest three receptor types: warmth receptors, pain receptors, and a "C receptor."

5. A close correlation between Weber increment and sensation increment has been made for warmth.

6. There are about 30 discriminable steps in the range of intensities from threshold to pain.

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THE PANCREATIC SECRETAGOGUE ACTION OF PRODUCTS OF PROTEIN DIGESTION

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Whether products of protein digestion contribute directly to the stimuli causing the intestinal phase of pancreatic secretion has not been determined. They are known to increase pancreatic secretion when taken by mouth (Dolinski, 1894; Bylina, 1911; Kobzarenko, 1915) but their effectiveness is generally attributed to the acid which they cause to be secreted by the stomach or to the water contained in their solutions (Babkin, 1914; Ivy, 1930). In a few experiments amino acids (Frouin, 1913; Arai, 1921) or peptones (Conheim and Clee, 1912) have been introduced directly into the intestine and stimulation of pancreatic secretion observed. The solutions used in some of these experiments were strongly acid. Neutral solutions were reported to have about the same effect as an equal amount of water. No technically satisfactory experiments have been reported. Many were done on anesthetized animals, generally the pH and freezing point of the solutions were unknown and in some the methods of collecting pancreatic juice were not such as to give reliable quantitative results. For this reason and in view of the recent observation (Crider and Thomas, 1940) that the water present in neutral, isotonic solutions fails to stimulate the pancreas when placed directly in the intestine, we determined to reinvestigate the problem.

METHODS. The arrangements for collecting pancreatic juice were the same as described in previous reports (Crider and Thomas, 1940; Thomas and Crider, 1940; Thomas, 1941). Four dogs were used but the quantitative data to be presented were obtained on three only, one having died before the work was completed. Each animal was provided with a cannulated duodenal fistula placed opposite the opening of the main pancreatic duct and the juice was collected through a rubber funnel held against the mucosa surrounding the duct. Appropriate control experiments proved that extraneous fluid (succus entericus?) collected by this method amounted to less than 1 cc. per hour and was not increased by injection of peptone or amino acids into the intestine. A cannulated gastric fistula was also made and effective drainage of the stomach was maintained during experimental periods. In one dog the bile duct was

transplanted into the stomach and in this and one other animal the accessory pancreatic duct was ligated at the time of operation. Much larger quantities of pancreatic juice were regularly obtained from the animals with accessory ducts ligated. Qualitatively, the results were uniform regardless of the type of operation.

Experiments were begun 18 to 24 hours after the last previous meal. Solutions were introduced into the intestine via a small ($\frac{1}{16}$ inch bore) rubber tube passed distally through the duodenal fistula to a point 6 inches to 12 inches below the pylorus.

The effect of peptone in the intestine was also tested on a few anesthetized animals but with uniformly negative results.

RESULTS. *The possible rôle of bile in the secretagogue action of peptone.* Very early in this study it became evident that introduction into the intestine of neutral, isotonic solutions of commercial peptones, certain amino acids, or protein digests prepared in the laboratory was followed regularly by an increase in the rate of pancreatic secretion. However, peptone in the intestine also stimulates the flow of bile (Bruno, 1899; Pavlov, 1910) and bile was thought by some to stimulate the flow of pancreatic juice (Mellanby, 1926). We therefore thought it advisable to postpone further study until the question of the pancreatic secretagogue action of bile could be investigated. Experiments of which a preliminary report has been made (Thomas and Crider, 1941) proved conclusively that, under our experimental conditions, bile in the intestine does not increase the flow of pancreatic juice.

Comparison of peptone with other stimuli. In this series of experiments the effect of injecting 20 cc. of a 5 per cent peptone (Bacto-Protone, Difco.) solution into the duodenum was compared with the effect of 10 cc. of N/10 HCl, or 40 to 60 cc. of distilled water, similarly administered, and with 4 "units" of a commercial secretin preparation (Pancreotest) given intravenously. The volume, specific gravity and total nitrogen of the pancreatic juice collected after stimulation were determined.

A consistent routine was followed in all the experiments. After the animal was prepared a single injection of one of the stimulating solutions was administered and after a convenient time, never more than one minute, a 10 ml. graduated cylinder was placed under the collecting tube and the secretion collected for 10 minutes. Again after a convenient time, generally one minute, a second injection of the same material was given and the above described procedure repeated until five or six samples had been obtained. Another stimulus was then substituted and the procedure repeated until the desired number of samples had been obtained with each stimulus. In a few experiments the secretion was collected for several minutes after feeding and studied to determine the properties of "psychic secretion."

The first few observations revealed the fact that although the properties of the secretion varied with the type of stimulus used, characteristic values were not obtained until at least two injections of the same stimulus had been given. Consequently, all samples obtained during the 10 minutes following the first injection of any stimulus were discarded and are not included in the reported data. Volume and specific gravity were determined on each sample and all but the first sample obtained during the use of a single type of stimulus were pooled for nitrogen determination. The order in which the various stimuli were used was varied from day to day.

The nitrogen determinations were made by a trained technician using the micro-Kjeldahl method. The method as used appeared to be subject

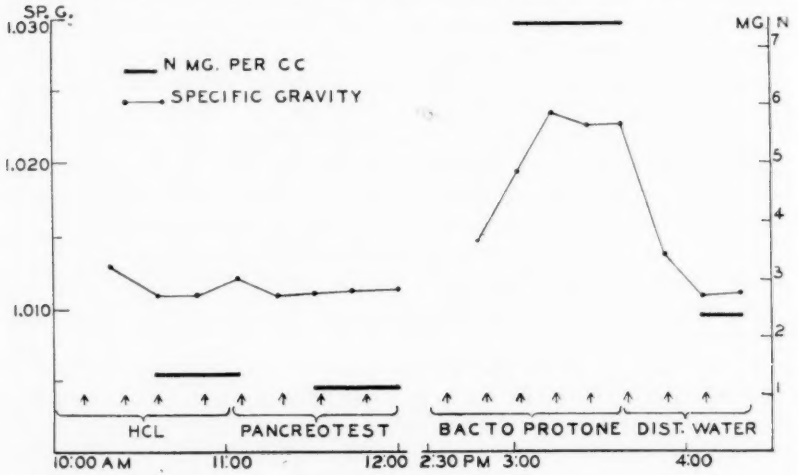

Fig. 1. Graphic protocol of a typical experiment on dog 2-40 showing specific gravity and total nitrogen (mgm. per cc.) of the pancreatic secretion obtained with various stimuli. The small arrows mark the moments at which injections were given.

to a maximum error of about 15 per cent. Specific gravities were measured by means of a 1 ml. specific gravity bottle.

The procedure and results of a typical experiment are illustrated graphically in figure 1. Average data obtained in all the experiments of this series are presented in table 1. It is evident that 20 cc. of peptone solution caused the production of a slightly larger amount of secretion than 40 to 60 cc. of distilled water. Also the secretion following peptone administration had a higher specific gravity and contained several times as much nitrogen per cubic centimeter as that caused by water, secretin, or HCl.

Comparison of various protein digests. This series of experiments was undertaken primarily to determine whether the capacity to stimulate the

secretion of pancreatic juice was limited to a few peptones or was characteristic of protein digests generally. As the work progressed it became evident that some digests were more effective stimuli than others so the data were arranged to bring out that fact as well. The experiments were conducted in the same manner as those in the group just preceding. Except in a few preliminary experiments, the total experimental time in any

TABLE 1

Amount and properties of pancreatic secretion produced by various stimuli

STIMULUS	DOG NUMBER	VOL. PER 10 MIN. SAMPLE	SP. G.	TOTAL N	TOTAL N	NUMBER OF SAMPLES
				mgm./cc.	*mgm./ sample*	
10 cc. N/10 HCl into duodenum	3-39	7.47	1.0119	1.54	11.5	4
	2-40	8.6	1.0103	1.38	11.8	6
	3-41	10.0	1.0099	0.84	8.4	6
Average		8.69	1.0107	1.28	10.56	
Secretin (4 units of pancreotest) intravenously	3-39	4.3	1.0133	—		4
	2-40	9.13	1.0113	1.12	10.22	3
	3-41	7.6	1.0111	0.42	4.19	4
Average		7.01	1.0118	0.77	7.20	
Dist. H_2O 40 to 60 cc. into duodenum	3-39	0.96	1.0112	—		2
	2-40	4.4	1.0110	2.38	10.38	2
	3-41	3.03	1.0127	2.66	8.06	6
Average		2.79	1.0116	2.52	9.22	
20 cc. 5 per cent bacto protone into duodenum	3-39	1.54	1.0365	16.96	26.12	5
	2-40	3.73	1.0224	6.94	26.6	13
	3-41	3.9	1.0174	5.9	21.09	13
Average		3.06	1.0254	9.930	24.61	
Feeding (psychic secretion)	3-39	—	1.0216	—	—	1
	2-40	—	1.0190	4.44	—	5
	3-41	—	1.0120	—	—	1
Average		—	1.0175	4.44	—	

one day was limited in this series to about three hours because it was found that the secretion obtained after prolonged stimulation had a lower specific gravity and contained less nitrogen than that obtained earlier.

The various substances used are listed in table 2. The commercial peptones were prepared in 5 per cent solution in 0.6 per cent sodium chloride solution. Freezing points were not determined but we know from pre-

vious experience that such solutions are approximately isotonic. The pH was determined for a sample of such a solution of each peptone; all were above pH 6.0. The digests prepared in the laboratory contained 4.0 mgm. of nitrogen per cc. and approximately 2½ per cent of solids. They were adjusted to between pH 6.8 and 7.2 with NaOH or HCl and made isotonic

TABLE 2

Amount and properties of pancreatic secretion produced by various protein digests

STIMULUS			SECRETION					RATIO, N SECRETED: N INJECTED
	Total N	Per cent proteose	Number of samples	Vol. per 10 min. sample	Sp. g.	Total N	Total N	
	mgm./cc.			*cc.*		*mgm./cc.*	*mgm./sample*	
Commercial products								
Witte's peptone	5.95	(high)	13	2.93	1.0249	9.60	24.01	0.201
Bacto protone	7.92	81+	31	3.06	1.0254	9.93	24.61	0.155
Neo peptone	7.42	25+	14	2.35	1.0209	8.20	17.71	0.133
Bacto peptone	8.26	6−	42	1.83	1.0226	8.86	14.47	0.092
Amino acid powder (Stearns)	6.46	0	11	1.08	1.0258	4.88*	6.29*	0.048*
Laboratory preparations								
Pepsin digest of casein	4.0	—	21	2.27	1.0218	7.526	15.541	0.194
Pepsin-trypsin digest of casein	4.0	—	22	1.96	1.0244	8.71	14.30	0.178
Proteose from neo peptone	4.0	100	24	2.39	1.0205	6.48	13.88	0.173
Proteose from intestinal contents	4.0	100	12	2.30	1.0202	6.39	13.27	0.164
Proteose from gastric contents	4.0	100	11	1.96	1.0199	7.00	12.55	0.156
P.T.A. digest of casein	4.0	(trace)	10	1.99	1.0233	5.5*	12.07*	0.15*

* Average based on data from 2 animals only.

(Δ between 0.5° and 0.65°C.) by addition of NaCl when necessary. The pepsin and pepsin-trypsin digests of casein were prepared as described in a previous article (Thomas and Crider, 1939). The "P.T.A." (pepsin-trypsin-alkali) digest was prepared by subjecting the pepsin-trypsin digest to prolonged boiling after saturating the solution with barium hydroxide.

The amino acid powder[1] gave a negative biuret test but was not guaranteed to be free of peptids. All solutions were injected in 20 cc. amounts.

The results are summarized in table 2. The figures presented are averages of the results obtained on the three dogs. There were consistent differences in the responses of the individual dogs, an estimate of which can be obtained from the data in table 1. The results recorded in the table show that all the preparations tried in this group of experiments were effective in causing secretion of a considerable amount (averaging 1 to 3 cc. in 10 min.) of pancreatic juice of high specific gravity and containing a large amount of nitrogenous material.

When preparations of uniform concentration in terms of nitrogen, such as the laboratory preparations, were used the stimulating activity of the various products may be compared by comparing the volume of the 10 minute samples or the total nitrogen secreted (mgm. per sample). For comparing these preparations with the commercial products, which were used in more concentrated solutions, the figures given in the last column of the table are presented. These values were obtained by dividing the figures for total nitrogen secreted (mgm. per sample) by those for total nitrogen injected (mgm. per 20 cc. of solution). Obviously, this method of comparison fails to take account of any influence which concentration alone independent of total quantity may have had on the effectiveness of the material.

It may be noted that differences in the effectiveness of the various peptone preparations as stimuli for the pancreas appeared chiefly as differences in the volume of secretion; specific gravity and total nitrogen (mgm. per cc.) showed little variation in the averages. We also noted that among the individual animals those yielding a larger volume of secretion in response to a given peptone stimulus produced proportionately less nitrogen in milligrams per cubic centimeter so that the total nitrogen secreted (mgm. per sample) in response to a uniform stimulus was remarkably similar from animal to animal. This fact is well brought out in the results with Bacto-Protone, presented in table 1.

Discussion. These experiments prove that various products of protein digestion are capable of acting in the intestine as powerful stimuli for certain functions of the pancreas, notably the secretion of nitrogenous solids. These products also increase the volume of the pancreatic secretion but in this respect they are inferior to HCl. It has also been shown that the secretagogue action of peptone solutions is not dependent on the water present nor on acid which they cause to be secreted. It is, therefore, a specific property of one or more of the products formed in the digestion of protein. What the active products are is difficult to determine with

[1] This material was kindly furnished gratis by Frederick Stearns and Co. of Detroit.

certainty but purified proteoses and some of the amino acids have been shown to be effective stimuli. On the other hand, the most abundant amino acids (glutamic acid and glycine) are known to be ineffective in neutral solution (Thomas and Crider, 1940) and some of the peptones and peptids may, therefore, also be inactive. However, the fact that eleven different preparations derived from various proteins and subjected to various degrees of digestion by different agents all exhibited the capacity to stimulate the pancreas indicates that this property is widespread among the products of protein digestion.

Among the commercial preparations the capacity to stimulate the pancreas appears to be inversely related to the relative completeness of digestion as indicated by the diminishing percentage of proteose. This fact suggests the attractive theory that only those products which require further digestion call forth an abundant secretion from the pancreas. Results obtained with the laboratory preparations do not seem to support this suggestion. For example, the proteoses were no more effective stimuli than the pepsin-trypsin digest of casein and of the three casein digests only the pepsin-trypsin-alkali (P.T.A.) digest appears, on the basis of data obtained from two animals, to have lost potency as a stimulus with the progress of digestion. There is reason to believe that data from the other animal would have diminished this apparent loss. The relative effectiveness as stimuli for the pancreas of the various classes of products of protein digestion must, therefore, remain undecided for the present.

The mechanism through which the peptones stimulate the pancreas has not been studied by the usual methods. Nevertheless, the properties of the secretion are such that it could not have been produced by the action of secretin alone. The only alternative mechanism known is nervous and it is significant that the properties of the secretion obtained by peptone stimulation are similar to those of "pilocarpine juice," "vagus juice," "psychic secretion" (see table 1) and the secretion following administration of acetylbetamethylcholine (mecholyl). We are, therefore, convinced that the peptones act through a nervous mechanism. If we are right we have, we believe for the first time, found a function of major physiological importance to be ascribed to the secretory nerve fibers that have long been known to supply the pancreas. We hope in the near future to begin experiments designed to trace the reflex pathway.

SUMMARY AND CONCLUSIONS

1. Products of protein digestion act in the intestine as stimuli for the external secretory function of the pancreas. Their effectiveness is not dependent on the coincident flow of bile into the intestine nor on the secretagogue action of water or acid.

2. The secretion produced by peptone stimulation has a higher specific

gravity and contains many times more nitrogen per cubic centimeter than that produced by water, acid or secretin.

3. There are wide variations in effectiveness as stimuli for the pancreas among various commercial peptones and protein digests prepared in the laboratory. Among commercial peptones the more effective products are those having the higher percentage of proteose.

4. The properties of the secretion are such that it could not be produced by secretin stimulation alone.

5. The secretion elicited by peptones resembles that caused by pilocarpine and other stimuli acting through or on the secretory nerves. Hence, the conclusion is drawn that the peptones act through a nervous mechanism.

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INFLUENCE OF PHYSICAL WORK ON PHYSIOLOGICAL REACTIONS TO THE THERMAL ENVIRONMENT

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Object of study. Previous studies from this laboratory have analyzed in some detail the reactions to various atmospheric conditions of the human body when at rest in a semi-reclining position (Winslow, Herrington and Gagge, 1937; Herrington, Winslow and Gagge, 1937; Gagge, Herrington and Winslow, 1937; Winslow, Herrington and Gagge, 1939; Gagge, Winslow and Herrington, 1938; Winslow, Herrington and Gagge, 1938; Winslow, Gagge and Herrington, 1939; Winslow, Gagge and Herrington, 1940). The purpose of the present investigation was to extend the observations made on resting subjects to subjects performing active physical work on a bicycle ergometer.

Conduct of experiment. The studies were carried out in an experimental booth which has already been described (Winslow, Gagge and Herrington, 1940) and by the same general techniques employed in our earlier work.

Air and wall temperatures were the chief atmospheric variables studied in the investigations here reported. They were accurately regulated and checked by records of air temperature and measurement of the brine temperature in the walls of the chamber (which determined mean radiant temperature) at twenty-minute intervals. The relation between brine temperature and mean radiant temperature was, of course, previously established by Vernon (1932) globe readings. Atmospheric humidity was maintained in general in the tests between 40 and 50 per cent saturation. Air movement in the chamber was measured by the hot-wire anemometer and was ordinarily 5 to 8 cm. per sec. In certain experiments, as noted, higher velocities were produced by the use of fans.

Two subjects (nos. VII and IX) were used in all tests, the same young men employed in earlier studies (Winslow, Gagge and Herrington, 1940).

The unclothed subject, after preliminary weighing, took his place on a bicycle ergometer of the recording electrodynamic type described by Kelso and Hellebrandt (1934), and began exercising at a predetermined load, measured on a recording voltmeter, and a fixed pedalling rate. Each experiment lasted for 90 minutes and computations of heat interchange were based on the period between $22\frac{1}{2}$ and $82\frac{1}{2}$ minutes from the beginning of the experiment.

The schedule of an experiment was as follows:

a. Rectal temperatures were recorded by a thermocouple inserted into the rectum at the beginning of the experiment and the measurement repeated every $7\frac{1}{2}$ minutes thereafter.

b. Skin temperature at fifteen representative points on the body surface was recorded at half-hour intervals by a Hardy thermopile.

c. Total metabolism (O_2 consumption) was determined twice with 15-minute samples, between $22\frac{1}{2}$ and $37\frac{1}{2}$ minutes and between $67\frac{1}{2}$ and $82\frac{1}{2}$ minutes after the beginning of the experiment, by means of a special adaptation of the Benedict-Roth apparatus, capable of handling rates up to ten times basal values (Gagge, 1941).

No determination of R.Q. was made since it appeared that the possible error in computing the calorie equivalent of the O_2 consumption on the basis of an assumed R.Q. of 0.83 would be very small. The typical exercise R.Q. has been shown to be affected by special diets and has been the subject of considerable dispute. Two recent studies (Christensen and Hansen, 1939a, 1939b) on subjects trained for ergometer work and living on a mixed diet have shown, however, that 0.83 is a representative value. Other data reported by the same investigators and also by Dill, Edwards, Bauer and Levenson (1931) have likewise shown that R.Q. is not affected by variations in environmental temperature over the range from 7 to 34°C. The inherent convenience of a record based on oxygen consumption alone was thought to outweigh, for our particular experimental aims, any minor increase in accuracy possible with gas analysis techniques.

d. The subject was weighed at the beginning of each experiment, after 15 minutes, after 45 minutes, and finally at the close of the experiment, on a sensitive platform scale placed adjacent to the bicycle.

Determination of convection constant. Of the five fundamental elements in thermal interchange, metabolic heat production and evaporative heat loss were thus obtained by direct observation. Convection and radiation interchanges are, of course, determined by air and wall temperatures, respectively, on the one hand and mean skin temperature on the other, which were recorded as indicated above. To determine the factor of heat loss by convection per degree difference between mean skin temperature and air temperature, the following series of experiments were conducted.

Wall temperatures were so regulated as to be approximately the same as mean skin temperatures, so that radiation interchange was practically nil. Air temperature, on the other hand, was varied over a range of 15°C. Observations were so made that the partitions applied to approximate "steady states"; hence thermal storage was usually insignificant. Under such circumstances, storage can be computed with reasonable accuracy from the changes which occur during the experiment in skin temperature and rectal temperature, giving the former a weight of 1 and the latter a weight of 2.

In the six experiments with each of two subjects, presented in table 1, it will be noted that work was limited to approximately 46.3 ± 0.2 kgm.-cal. per hour and the corresponding metabolism ranged between 297.0 and 328.2 kgm.-cal. Mean radiant temperature and mean skin temperature never differed by more than 1.6° and radiation heat interchanges, therefore, varied only from + 7.6 to − 13.4 kgm.-cal. Storage exceeded 9 kgm.-cal. in only two instances and in only one of these exceeded 13 kgm.-cal. This was because the skin temperature of the subjects never changed more than about 1° and the rectal temperature never more than 0.5°. Thus, the convection loss, computed from the algebraic sum of metabolism, work, evaporation, storage, and radiation was chiefly determined by the large measured factors of metabolism and evaporation.

TABLE 1

Experiments conducted for the determination of the convection constant

SUBJECT AND EXPERIMENT	TEMPERATURE, °C.			HEAT INTERCHANGE, KILOGRAM-CALORIES PER HOUR					
	T_A*	T_W*	T_S*	M†	W‡	E§	R¶	S‖	C**
VII-77	15.7	30.2	31.7	297	−46	−73	−10	−6	−161
78	16.7	32.1	32.3	315	−46	−127	−2	−9	−132
79	19.6	30.8	32.2	313	−46	−114	−11	−3	−139
80	23.3	31.2	32.8	304	−46	−153	−12	−10	−82
81	28.3	32.3	33.9	316	−46	−193	−12	−10	−54
82	32.1	34.3	34.3	316	−46	−234	0	−9	−26
IX-63	17.1	32.4	31.3	307	−46	−118	7	3	−153
64	17.9	31.4	31.8	322	−46	−106	−3	4	−171
65	21.3	32.7	31.6	300	−46	−135	7	3	−124
66	24.1	31.8	32.0	309	−46	−202	−1	6	−64
67	27.8	31.9	31.8	315	−46	−216	1	−3	−49
68	32.4	33.6	33.4	328	−47	−287	1	2	2

* T_A, T_W, and T_S, air temperature, mean radiant wall temperature, and mean skin temperature.

† M, metabolism.

‡ W, work, as measured from the calibration of the ergometer.

§ E, evaporative heat loss.

¶ Computed on assumption that radiation area is 70 per cent of total body area.

‖ Computed from changes in skin temperature and rectal temperature.

** Computed from $M - W - E \pm S \pm R$.

In the upper part of figure 1 we have plotted the convection loss, as thus estimated, against the difference between mean skin temperature and air temperature for each experiment. The slope of this curve gives a convection constant of between 10 and 11 kgm.-cal. of heat loss for each °C. difference in temperature between skin and air. It will be noted that the points fall about the mean slope as drawn except in the case of one of the

two coldest experiments for subject VII. This departure is in accord with our earlier studies which show that under cold conditions considerable storage (cooling of body tissues) occurs without being immediately mirrored in fall of skin and rectal temperature.

When the convection constant is computed for the individual as a unit, it is apparently the same for both subjects. If computed per unit surface area—as in our earlier work on subjects at rest—the constant is 5.3 for subject VII (a large individual) and 6.7 for subject IX (a small individual). Since, in the case of a subject actively pedalling on a bicycle, convection loss is largely determined by the movement of the legs it is not surprising that, when subjects differ in size, the over-all heat loss from the whole body gives more consistent results than that computed for a standard area. In a subject at rest, both metabolism and processes of heat loss are adjusted to total surface area but this is not the case in exercise where the level of heat production is arbitrarily set by the severity of the task. We shall, therefore, present our data in general on the basis of the total subject and not—as in much of our previous work—per square meter of body surface.

Influence of pedalling rate and air movement on the convection constant. For rough comparison with results on resting subjects we may take a convection constant of 5.9 per square meter per hour (per °C. difference in temperature between skin and air) as representing an approximate mean value for the two subjects under working conditions.

In our earlier work on resting subjects in a semi-reclining position we found the convection constant to be $1.0\sqrt{V}$ where V is the velocity of air movement in centimeters per second (Winslow, Gagge and Herrington, 1940). In the present series of studies four tests were made on each subject in a sitting posture on the bicycle but at rest without pedalling. The constants obtained are shown in table 2, for the whole subject, and per square meter, and also the component which gives the constant when divided by $\sqrt{V}$.

The mean of these eight values is $0.8\sqrt{V}$ which means that the rate of convection loss for the subject sitting up at rest is slightly less than in the case of the reclining subject. This is in accord with what we would expect. The reclining subject has his long diameter in a more nearly horizontal position than the sitting subject; and the more nearly vertical is the position of the heated cylinder, the less will be the rate of convection loss.

It will be noted that the constants for convection loss from the whole body of the subject in table 2 for low air velocities are less than one-third those derived from table 1. The difference is obviously due to increased convection due to the movements (particularly of the legs) involved in pedalling. To measure this factor and to determine its relation

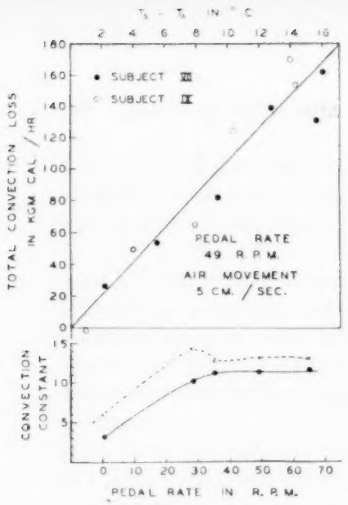

Fig. 1

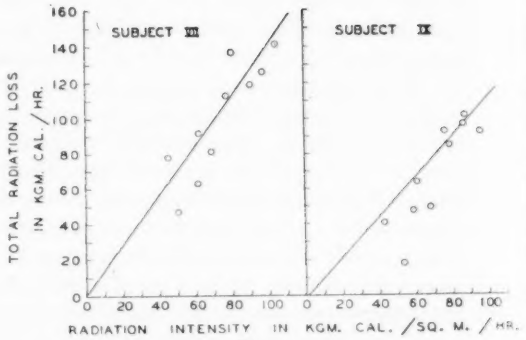

Fig. 2

Fig. 1. Upper graph. Relation of heat loss by convection to difference between skin and air temperatures (basic curve for determination of convection constant).

Lower graph. Relation of convection constant to varying pedalling rates.

Fig. 2. Relation of heat loss by radiation to intensity of radiative demand of the environment (basic curve for determination of radiation area).

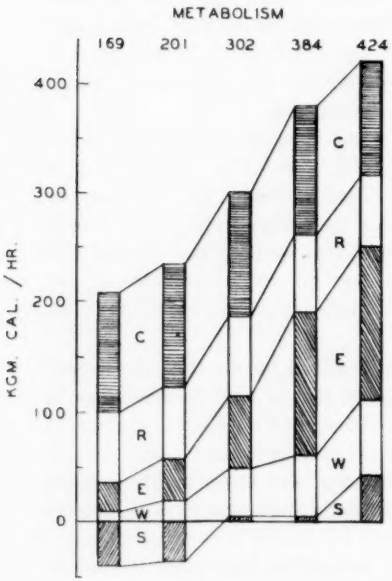

Fig. 3

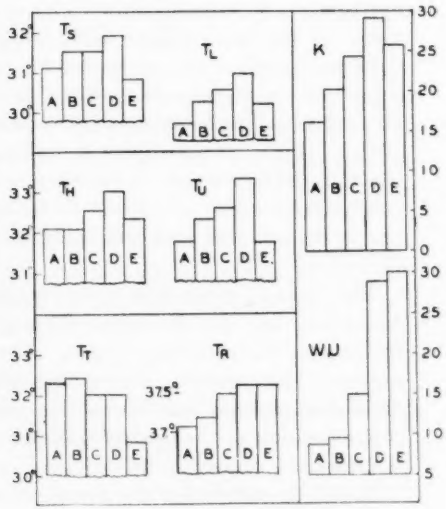

Fig. 4

Fig. 3. Variation in heat interchange by various avenues with rate of metabolism (as determined by varying rate of work).

C = convection heat loss, R = radiation heat loss, E = evaporative heat loss, W = energy consumed in work, S = storage (values below zero denote body cooling).

Total height of column equals metabolism.

Fig. 4. General and regional skin temperatures, conductance, rectal temperatures, and wetted area for five different rates of metabolism. Metabolic value: A, 169; B, 201; C, 302; D, 384; E, 424 kgm.-cal. per hour.

to air movement we conducted 50 experiments, with varying pedalling rate, 30 at normal low air velocities and 20 with high air velocities (17–23 cm./sec.). The total amount of work performed (and the related metabolism) were approximately the same, the different pedalling rates being

TABLE 2

Convection constants for subjects at rest in sitting posture on the bicycle

AIR VEL.	SUBJECT VII			SUBJECT IX		
	$C/\Delta T$	$\frac{C}{\text{sq. M} - \Delta T}$	$\frac{C}{\text{sq. M} - \Delta T - \sqrt{V}}$	$C/\Delta T$	$\frac{C}{\text{sq. M} - \Delta T}$	$\frac{C}{\text{sq. M} - \Delta T - \sqrt{V}}$
cm./sec.						
4.1	2.5	1.2	0.6	3.2	2.0	1.0
5.1				3.6	2.3	1.0
6.1	3.4	1.7	0.7			
17.8	7.4	3.7	0.9	5.0	3.1	0.7
19.3	5.6	2.8	0.6			
20.3				5.5	3.4	0.8

TABLE 3

Total convection constant for varying pedalling rates at low and high air movement

SUBJECT	AIR MOVEMENT									
	4-8 cm./sec.					17-23 cm./sec.				
	Pedalling rate, R.P.M.									
	0	28-29	34-36	48-50	63-68	0	28-29	34-36	48-50	63-68
VII	2.5	10.3	11.2	17.8	13.1	7.4	15.3	13.4	18.1	13.0
	3.4	10.8	11.2	9.6	11.9	5.6			13.8	
		11.4	11.8	11.7	12.0				10.3	
				12.2					17.1	
				11.5						
				11.4						
IX	3.2	7.2	12.7	9.7	11.8	5.0	16.6	12.6	13.6	12.4
	3.6	10.8	10.1	10.1	11.5	5.5	14.4	12.2	9.4	13.4
		11.5	11.0	10.4	9.7		11.4	12.6	9.2	14.1
				13.5					12.9	
				9.2						
				10.8						
Mean.....	3.2	10.3	11.3	11.5	11.7	5.9	14.4	12.7	13.1	13.2

regulated by decreasing or increasing the resistance of the bicycle. We have expressed all results in terms of total body area since—as pointed out above—this is the most consistent measure of convection loss under the conditions of the experiment. We have also added one column of table 2 for comparison.

It will be noted (table 3) that the process of pedalling even at the low

rate of 28 to 29 revolutions per minute, nearly tripled the convection constant and further increase did not materially change the picture. The lack of further increase with more rapid pedalling is perhaps not surprising, since the legs carry a blanket of warmed air with them and movement at more than a certain rate might naturally be ineffective.

The mean results of table 2 plotted at the bottom of figure 1 confirm our constant of 11 for the pedalling subject at low air movement; and this constant for an air movement of about 5 cm. per sec. is equivalent to the constant which would be observed for a subject at rest with an air movement of 30 to 40 cm. per sec. This latter factor is an equivalent measure of the increase in convective heat loss due to the movements of the body.

So far as the influence of air movement itself is concerned, we note that an increase from 5 cm. to 20 cm. for the resting subject doubled the convection loss which accords with the square root formula.

In the case of the pedalling subject, a similar increase in external air movement only raised the convection constant from 11 to 13. If, however, we recall that the actual combination of air movement about the body and body movement through the air is 30 + 5 in the first case, and 30 + 20 in the second case, the convection constant should be raised a little less than 20 per cent by the additional air movement—as is the case. For our standard pedalling rate, the convection constant is about 11, and this value (rather than the 10.5 indicated in the upper part of the graph) has been used in our later computations.

Determination of the radiation area. Having determined the convection constant, a series of 10 experiments were made with each of our two subjects to determine their radiation area. The experiments were so set up that air temperature varied but little (from 18.4 to 22.4°C.) and that storage (estimated from change in skin and rectal temperature) was small (from +20 to −11 kgm.-cal.) in relation to total heat interchange. Skin temperature changed less than 1°C. (except in three cases, where the changes were less than 1.2°, respectively). Rectal temperatures changed less than 0.3° (except for one case where it reached 0.55°). Metabolism varied from 347 to 374 for subject VII and from 319 to 343 kgm.-cal. for subject IX. The work performed varied from 48 to 55 kgm.-cal.

It will be noted from table 4 that with both subjects, radiation loss dropped rapidly between experiment 12 and experiment 13, when wall temperatures began to exceed 15°C. In the case of subject VII, active increase in sweat secretion took place from experiment 8 on, while the increase began with subject IX in experiment 11 when the radiant temperature reached about 14°C. Clearly, the subjects were in the phase of evaporative regulation throughout all these experiments, the increasing sweat secretion just balancing a tendency to decreased radiation as conditions became progressively warmer.

For determination of the radiation area, the total radiation loss for each subject has been plotted in figure 2 against "radiation intensity"—the heat loss per square meter which would be expected from Stefan's Radiation Law for the recorded difference between skin temperature (T_S) and mean radiant temperature of the surroundings (T_W). The slope of the graphs gives the radiation area which amounts to 72 per cent of the total

TABLE 4

Experiment conducted for the determination of the radiation area

SUBJECT AND EXPERIMENT	TEMPERATURE, °C.			HEAT INTERCHANGE, KILOGRAM-CALORIES PER HOUR						RELATION INTENSITY, R‡
	T_A	T_W	T_S	M	W	E	C	S*	R†	
VII- 8	18.5	8.6	29.7	350	−50	− 35	−119	−5	−141	−104
9	18.4	10.8	30.3	347	−50	−53	−129	+11	−126	−97
10	18.9	12.3	30.2	351	−51	−68	−120	+7	−119	−90
11	19.9	14.3	30.1	357	−51	−52	−108	−9	−137	−80
12	19.8	15.2	30.3	372	−52	−100	−111	+8	−113	−77
13	20.6	18.0	31.4	366	−55	−118	−114	+8	−81	−67
14	21.1	19.4	31.4	350	−50	−104	−109	+5	−92	−62
15	21.3	20.4	31.9	374	−51	−141	−112	−7	−63	−61
16	22.4	22.6	31.9	351	−51	−145	−101	−7	−47	−50
17	22.3	23.1	31.6	358	−48	−143	−99	+10	−78	−45
IX- 8	18.9	9.3	28.8	330	−51	−83	−105	+1	−92	−96
9	19.3	11.5	29.2	328	−48	−77	−105	+3	−101	−88
10	19.3	11.7	29.2	330	−50	−70	−105	−9	−96	−87
11	19.6	13.8	29.5	343	−50	−100	−105	−4	−84	−79
12	19.9	15.1	30.0	340	−51	−101	−107	+11	−92	−76
13	20.2	16.8	30.1	331	−50	−142	−105	+15	−48	−68
14	20.4	17.9	29.7	328	−53	−122	−98	+8	−63	−61
15	20.6	19.1	30.4	331	−50	−150	−104	+20	−47	−59
16	21.4	20.6	30.7	319	−50	−155	−98	+1	−17	−53
17	22.4	22.7	30.9	337	−50	−159	−90	+2	−40	−43

* Computed from changes in skin temperature and rectal temperature.

† Computed from $M - W - E \pm S - C$.

‡ Calculated from Stefan's Radiation Law,

$$R = 4.92 \times 10^{-8} (T_W^4 - T_S^4).$$

surface area for subject VII and 67 per cent for subject IX. It will be noted that under hot conditions for subject IX, three of the points fall below the graph which—as shown in our earlier communications—indicates that the sweat is running off without evaporating and thus is not exerting its cooling effect upon the body.

It appears then that the effective radiation area for a subject pedalling on a stationary bicycle is about 70 per cent of the total surface area, as was found to be the case for the resting subject (Winslow, Gagge and Herrington, 1940).

In all future computations of the present paper, where the pedalling rate is 48, we shall use the convection constant of 11, and compute radiation as a 70 per cent radiation area, estimating storage by difference from the formula

$$M - W - E \pm C \pm R = S.$$

The influence of variations in work and metabolism upon thermal interchange. The major difference between the conditions here discussed (aside from the influence of body movement upon the convection constant) is the performance of physical work and the accompanying higher metabolism. A series of eight experiments were conducted with each of our two subjects in which the amount of work performed was the only variable, to measure the influence of these factors more closely.

In these experiments, air and wall temperatures were both held between 20.6 and 21.6°C. Under these conditions the mean skin temperatures of the subjects varied between 30.3 and 32.6°C. Subject VII had a skin temperature between 31.1 and 31.6°C. in all but two of the high-work experiments in which T_S rose to 32.6°. Subject IX had a T_S varying from 30.3 to 31.7°C., corresponding to his higher evaporative heat loss. The data with regard to thermal interchange are summarized in table 5.

It will be noted that when performing work at a rate of 8 kgm.-cal. per hour, subject VII had a metabolism of about 200, compared with a resting figure for this subject while on the bicycle of 140; subject IX had a metabolism of about 150, compared with a resting figure of 110. When performing work of 19 kgm.-cal., the metabolism for subject VII changed very slightly while for subject IX it rose to nearly 200. At a work-rate of 45 kgm.-cal. both subjects showed a metabolism of about 300. At 57 kgm.-cal., both metabolisms rose to nearly 400; and at 70 kgm.-cal. of work to over 400. Omitting experiment VII-62 where metabolism was abnormally high, the efficiency of the work performed varied between 0.20 and 0.30 and averaged 0.24.

Convection and radiation showed no significant relation to work as must necessarily be the case since skin temperatures remained so nearly uniform. Storage was positive (cooling of body tissues) with work rates of 8 and 19 kgm.-cal. It was negative at higher work rates, except in experiments VII-63 and IX-51.

Since no great difference in reaction was manifested by the two subjects and since the number of experiments with each subject was the same, we have averaged all results for both subjects at a given work rate and presented them in figure 3. Positive storage (cooling of body tissues) has been plotted below the base-line, negative storage, above. The total height of the combined bars above the base line corresponds to the metabolism.

It will be noted that as work (and metabolism) increased, evaporative heat loss increased *pari passu* but not at so great a rate (except for the interval between 45 and 57 kgm.-cal. where evaporation almost exactly kept up with the increased thermal load); therefore, storage passes gradually from a positive to a negative value.

It is of interest to note how fallacious is the habit of expressing heat-interchange by various avenues as percentages of metabolic heat production. With subjects at rest (and equal wall and air temperatures) radiation and convection heat losses are nearly equal. With a pedalling subject, convection loss is about 70 per cent in excess of radiation loss as a result

TABLE 5

Thermal interchange with varying work loads and related metabolism (pedalling rate held constant at 38 R.P.M.)

SUBJECT AND EXPERIMENT	TEMPERATURE, °C.			HEAT INTERCHANGE, KILOGRAM-CALORIES PER HOUR					
	T_A	T_W	T_S	M	W	E	R	C	S
VII-62	21.1	21.4	31.6	210	−8	−29	−76	−116	19
66	21.5	21.6	31.2	169	−8	−26	−72	−107	44
63	20.8	21.3	31.5	211	−19	−29	−76	−118	31
67	21.3	21.5	31.1	203	−19	−40	−71	−108	35
64	21.2	21.4	32.6	295	−45	−59	−83	−125	17
68	20.9	21.1	31.3	300	−45	−42	−76	−114	−23
65	21.2	21.3	32.6	397	−57	−92	−84	−125	−39
69	21.1	21.2	31.3	439	−70	−105	−75	−112	−77
IX-48	21.0	21.3	30.8	144	−8	−27	−56	−108	55
52	21.4	21.6	30.6	153	−8	−34	−53	−101	43
49	21.1	21.4	31.7	201	−19	−38	−61	−117	34
53	21.1	21.4	31.5	191	−19	−43	−59	−114	44
50	20.6	21.1	31.4	317	−45	−86	−61	−119	−6
54	20.9	21.3	30.8	297	−45	−82	−56	−109	−5
51	20.9	21.2	31.0	371	−57	−169	−58	−111	24
55	21.0	21.1	30.3	408	−70	−172	−54	−102	−10

of the movement of the limbs. Under such conditions, air temperature is much more important than mean radiant temperature in determining comfort. Furthermore, as seen from table 5, evaporative heat loss increases threefold for subject VII with increasing work and more than five times with subject IX, while convection and radiation losses remain fairly constant.

Reactions of working subjects at various operative temperatures. The operative temperature has been defined in our earlier studies as the temperature representing the combined influence of air and surrounding surfaces on heat losses from the body. The actual air temperature and mean radiant temperature must be duly weighted for radiation and convection

constants, respectively. To avoid this computation, we have conducted 19 experiments for each subject in which air temperature and mean radiant temperature were nearly identical, almost never differing by more than 1°C., and at a constant air movement of 6 to 8 cm. per sec. Under

TABLE 6

*Basic data at various operative temperatures with wall and air temperature equal**

SUBJECT AND EXPERIMENT	DATE	T_O†	T_S†	T_H†	T_U†	T_T†	T_L†	T_R†	M‡	W‡	E‡	R‡	C‡	S‡	K§	W¶	VOTE‖
VII-72	1/15/41	12.4	27.9	29.4	28.6	29.3	26.4	37.4	305	−47	−26	−109	−171	46	15.8	5.6	1
71	1/14/41	15.5	29.6	30.6	31.1	30.8	27.6	37.5	328	−46	−45	−102	−155	20	18.9	9.5	2
34	9/10/40	15.6	30.4	30.0	31.3	31.6	29.1	37.6	320	−45	−45	−108	−162	40	21.6	10.3	4
33	9/ 6/40	15.7	29.9	30.1	30.4	31.2	28.6	37.2	313	−46	−38	−103	−155	29	20.0	8.5	2
47	11/ 1/40	15.8	29.8	31.1	31.0	31.2	28.0	37.9	335	−46	−48	−100	−155	14	18.5	10.4	2
46	10/31/40	16.1	30.0	31.3	30.8	31.8	28.1	37.7	315	−46	−46	−100	−153	30	19.2	9.6	3
70	1/13/41	18.7	31.2	31.6	33.4	31.7	29.6	37.4	303	−46	−45	−93	−135	16	21.8	8.2	3.5
50	11/ 7/40	20.2	31.2	31.8	32.9	31.9	29.7	37.6	317	−46	−49	−82	−109	−31	18.5	9.4	4
73	1/16/41	20.8	31.8	32.8	33.1	32.6	30.5	37.9	314	−47	−48	−82	−122	−15	18.7	10.6	4.5
15	7/16/40	20.9	31.9	31.9	32.6	32.5	30.8	37.7	374	−51	−141	−86	−117	−21	21.9	30.0	4.5
68	1/ 7/41	21.0	31.3	32.2	32.2	31.6	30.4	37.4	300	−45	−42	−76	−114	−23	18.7	8.0	3.5
57	11/27/40	21.2	31.8	32.3	33.2	31.9	30.8	37.7	380	−46	−54	−77	−120	−83	24.4	10.1	4
64	12/19/40	21.3	32.6	33.2	33.7	32.8	31.6	38.0	295	−45	−59	−84	−125	18	24.4	10.5	4.5
16	7/17/40	22.5	31.9	32.1	32.7	32.8	30.7	37.7	351	−51	−145	−70	−105	20	27.2	30.0	4
17	7/18/40	22.7	31.6	31.6	32.6	32.0	31.0	37.5	358	−48	−143	−64	−102	1	26.0	27.6	5
74	1/17/41	24.3	33.4	33.9	34.4	34.1	32.3	37.7	307	−46	−85	−71	−99	6	30.7	14.5	4.5
75	1/20/41	26.8	33.4	33.6	34.2	33.7	32.9	37.7	317	−46	−117	−51	−74	−29	27.6	20.8	4.5
76	1/21/41	31.6	34.7	35.2	35.0	34.6	34.4	37.8	320	−46	−200	−25	−34	−15	41.3	33.8	4.5
6	2/ 5/41	33.2	34.3	35.1	34.6	34.2	34.0	37.6	316	−46	−234	−0	−24	−12	38.2	40.2	5
IX-58	1/15/41	12.4	28.4	30.5	30.1	31.2	25.2	37.8	310	−46	−44	−90	−176	46	20.6	11.8	2
57	1/14/41	15.7	29.4	31.1	31.0	31.6	26.8	37.4	307	−47	−63	−79	−151	33	22.9	16.3	3
33	11/ 1/40	15.8	29.6	31.3	30.9	31.6	26.9	37.7	303	−46	−48	−78	−153	22	21.5	13.3	3
23	10/31/40	16.1	29.8	31.9	30.6	31.8	27.5	37.9	316	−46	−49	−78	−151	8	21.5	13.5	3
32	9/10/40	15.6	29.7	30.8	30.7	31.6	27.6	37.3	288	−46	−49	−81	−154	42	23.4	15.1	3
22	5/ 9/40	16.2	30.2	31.4	31.1	31.7	28.3	37.4	322	−46	−69	−82	−152	27	26.2	19.0	4
56	1/13/41	18.4	31.3	32.4	32.2	32.3	29.7	37.7	310	−46	−55	−76	−140	7	26.5	12.7	4
15	7/18/40	19.9	30.4	32.3	31.8	31.3	30.5	37.9	331	−50	−150	−66	−108	43	27.0	46.3	4
30	11/ 7/40	20.2	31.3	33.1	32.5	32.5	29.6	37.8	329	−46	−76	−66	−120	−21	25.3	18.3	4.5
59	1/16/41	20.6	31.0	32.9	32.7	32.0	29.1	37.6	329	−46	−101	−61	−116	−5	26.4	23.8	4
50	12/19/40	20.9	31.4	32.4	32.6	32.2	30.2	37.4	317	−45	−86	−61	−119	−6	27.7	20.8	4
16	7/17/40	21.0	30.7	32.0	31.6	31.8	29.3	37.4	319	−50	−155	−59	−102	47	29.4	46.0	4
54	1/ 7/41	21.1	30.8	32.1	31.8	31.4	29.6	37.2	297	−45	−82	−56	−109	−5	24.1	20.3	4
44	11/27/40	21.3	30.9	32.2	32.3	31.9	29.2	37.5	308	−46	−117	−54	−110	19	26.7	28.9	4
17	7/18/40	22.6	30.9	32.9	32.1	31.4	29.5	37.8	337	−50	−159	−49	−94	15	27.2	45.3	4.5
60	1/17/41	23.9	31.7	33.4	33.2	32.1	30.4	39.6	335	−46	−158	−47	−87	3	30.9	38.8	4
61	1/20/41	26.4	31.8	33.3	32.9	31.9	30.8	37.5	298	−46	−187	−31	−63	29	31.0	40.0	4
62	1/21/41	29.4	32.9	34.1	33.3	31.8	31.9	37.6	323	−46	−232	−22	−40	17	39.2	54.5	4.5
6	2/ 5/41	33.0	33.4	35.3	34.2	32.4	33.9	37.7	328	−47	−287	1	−11	16	43.3	67.0	5

* Relative humidity, 40–50 per cent saturation; air velocity, 5–8 cm. per sec.; pedalling rate, 48 R.P.M.

† Operative temperature, mean skin temperature, temperatures of head, upper extremities, trunk and lower extremities, rectal temperature, °C.

‡ Metabolism, work, evaporative heat loss, radiation loss, convection loss, storage.

§ Conductance in kilogram-calories per square meter per hour per °C. difference between T_R and T_S.

¶ Degree of wettedness, in kilogram-calories per square meter per hour per centimeter of Hg vapor pressure difference between skin and air.

‖ Vote of subject as to thermal comfort on a scale of 5, 1 being cold and 5 hot.

these particular circumstances, the mean of the two temperatures is, for all practical purposes, the operative temperature itself; and the pertinent data for these 38 experiments are presented in table 6. In all instances, relative humidity was moderate (50–60 per cent) and the work done (at a standard pedalling rate of 48–50 revolutions per minute) varied only from 45 to 51 kgm.-cal. per hour.

Metabolism and storage. It will be noted from table 6 that metabolism was generally between 290 and 340 kgm.-cal. for both subjects. Subject IX showed one low value of 288 and a maximum value of 335 kgm.-cal. Subject VII had a minimum value of 295 and four abnormally high values of 351, 358, 374 and 380, respectively. With neither subject, however, was there any relation between metabolism and operative temperature.

Storage values were also fairly uniform, ranging from −31 (heating of the body tissues) to +50 (cooling of the body tissues) in all but one instance. In one experiment with subject VII (a pyknic subject) a very high value for negative storage of −83 was recorded. This was in experiment VII-59, which was the experiment with a metabolism of 380. Omitting this single abnormal figure we find that mean storage values varied as below.

	To		
	Under 17°	18°-22°	Over 22°
Storage, Subject VII	+33	−10	−5
Subject IX	+30	+9	+16

Thus, for both subjects, body cooling was definitely present in every experiment at all operative temperatures below 17°C. At operative temperatures of 18° and above, subject VII (the pyknic) showed a general tendency at end of experiment toward body heating during exercise, subject IX (a slender subject) a slight tendency to body cooling.

The mean, semi-reclining metabolism of these two subjects is about 86 Calories, so that mean work metabolism here is about 3.7 resting basals. At 31°C. we have usually said it takes 4°C. to dissipate body heat: 3.7 × 4° equals 14.8°; 31° minus 14.8° equals 16.2°, which is very close to our 17°C. neutral condition. It appears as though the equilibrium point for a given work-rate might almost be computed by multiplying the working metabolism expressed as a multiple of basal metabolism by 4 and subtracting the value obtained from 31°.

Heat loss by radiation and convection. Heat loss by both radiation and convection, of course, steadily increased with falling operative temperature. It is interesting to note from table 6 that convection loss for the two subjects was essentially identical at any given operative temperature.

For any given operative temperature, however, subject VII lost about 20 more kgm.-cal. than subject IX by radiation. This, of course, merely illustrates the fact that under working conditions—where the rapid movement of the legs is the main factor in convection loss—body build and body size have little influence on the magnitude of the convection but they still play an important rôle in determining the magnitude of the radiation loss.

Skin temperature, rectal temperature and conductance. It will be noted, from table 6, that mean skin temperature for both subjects falls with decreasing operative temperatures but a trifle more rapidly for subject VII, the pyknic subject, because his skin temperature was higher under hot conditions. Thus, for subject VII the mean T_S fell from 34.3° at a T_O of 33.2° to a value of 27.9° at a T_O of 12.4°. For subject IX, the corresponding T_O values were 33.4° and 28.4°. In each case a decrease of 20° in operative temperature caused a fall of from 5° to 6.5° in mean skin temperature. This is much the same reaction as that of the resting subject (Herrington, Winslow and Gagge, 1937).

For comparison, we have averaged the data for each half-degree temperature interval and have compared the results, in figure 5, with those obtained for subjects IX and VII in a resting position as reported in an earlier paper (Winslow, Herrington and Gagge, 1937) for both mean skin temperature and conductance.

It is most interesting to note that the variations of both skin temperature and conductance with falling operative temperature are almost identical for the resting and the working subject. Each individual has his own level of skin temperature under warm conditions, that for subject VII being high and for subject IX being low. Under cold conditions all fall close together. With subject VII—observed in both series—skin temperature and conductance (which varies with vasodilatation) were actually lower at a given high operative temperature under work conditions than at rest. This indicates a surprisingly perfect adaptation to the enormously increased load of heat production under conditions of vigorous physical work.

Furthermore, the fall of skin temperature for the main regions of the body surface was much the same in the working and resting state. We had thought that, on account of the large amount of heat produced in the lower extremities, the skin temperatures of this region might fall less rapidly under working conditions; but such was apparently not the case.

It will be noted that, in general, the regional skin temperatures for the resting subjects were considerably higher than for the working subjects (the only exception being that of the upper extremities under cool conditions). This is, of course, due to the higher evaporative heat loss of the working subjects. It may be noted that under very hot conditions the skin tem-

peratures of the resting subjects dip sharply down as their sweat secretion sets in. The individual regions of the body exhibit the same general trends in all cases.

Rectal temperature, on the other hand, shows a marked difference between the working subject and the resting subject, as indicated in figure 5.

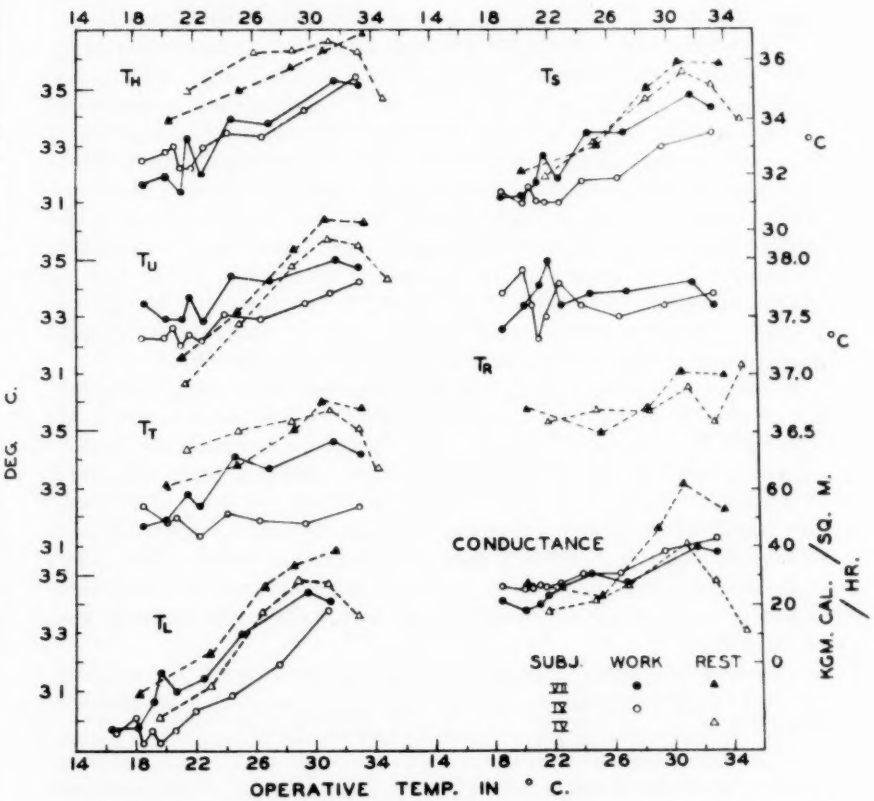

Fig. 5. Relation of general and regional skin temperatures, conductance, and rectal temperatures to operative temperatures.

For resting subjects, the rectal temperature is in the neighborhood of 36.9° at operative temperatures above 30° and falls to about 36.6° in a cooler environment. The rectal temperature of the working subjects, however, remains at about 37.6° throughout the whole range of operative temperatures. A difference of 1° in rectal temperature is highly significant and is no doubt associated with the high metabolism of the working subject.

Evaporation and wetted area. The evaporative heat loss clearly represents the mode of thermal adaptation to working conditions and the results in this respect are, therefore, of special interest. It will be noted from table 6 that at operative temperatures below 19°, evaporative heat loss is always relatively low (below 70 kgm.-cal.). Between 19° and 25° T_o it varies very widely (from 40 to 160) and apparently at random. Above 25° it is always high and rises steadily with further rises in T_o.

The factor which governs the adaptive process of evaporation (Gagge, 1937) is the degree of wettedness of the skin surface (w_μ). This factor again shows low values (under 20) below 19° T_o, and high and progressively increasing values above 25° T_o—with wide variations between 20° and 25°. We were puzzled at first by the sharp and apparently meaningless variations in the middle range—until it was noted that the low values had always occurred in winter, high values in summer. In the light of earlier studies (Winslow, Herrington and Gagge, 1938) which showed a seasonal variation in the efficiency of the evaporative mechanism this seemed to offer a reasonable explanation of the apparent discrepancies. In figure 6 we have, therefore, plotted separately the data for each subject at each season—with earlier data for resting subjects for comparison.

It is clear from this graph that with both subjects VII and IX in winter the process of active sweat secretion in the working subject sets in at about 20° T_o. In summer, both subjects react more promptly (somewhere between 16° and 20°) and at 20° have reached a fairly high level of evaporative wettedness. For the resting subject, the onset of evaporative regulation is reached only above 30°. At all points, subject IX (the leptosome) exhibits a higher evaporative rate than subject VII. This is, no doubt, because the slender subject, when working at the same high metabolic rate as the stout subject, must balance the higher radiation loss of the latter.

Sensations of comfort. At the close of each experiment, the subjects expressed their temperature sensations on a scale in which 1 represented "cold," 2, "cool," 3, "ideal," 4, "warm," and 5, "hot."

At operative temperatures below 15°C. the mean vote of both subjects was 1.5; at temperatures between 15° and 17° it was 2.9; at temperatures between 17° and 22°—and also at temperatures above 22°—the mean vote was 4.1. Thus, the ideal sensation for the working subject was at about 16°C., as compared with 28° for the resting subject (Winslow, Herrington and Gagge, 1937). On the other hand, the sensation of thermal comfort for the working subject occurs at a slightly lower skin temperature (31.3°) than for the resting subject (32.8°). Thus, it appears that an increase of metabolism from 100 to 300 kilogram-calories is equivalent to a difference of 12° in operative temperature, so far as its influence on comfort is concerned.

Reactions of subjects working at various rates. To check up on the observed phenomena from a slightly different angle, we may analyze physiological reactions for two series of experiments with each subject under

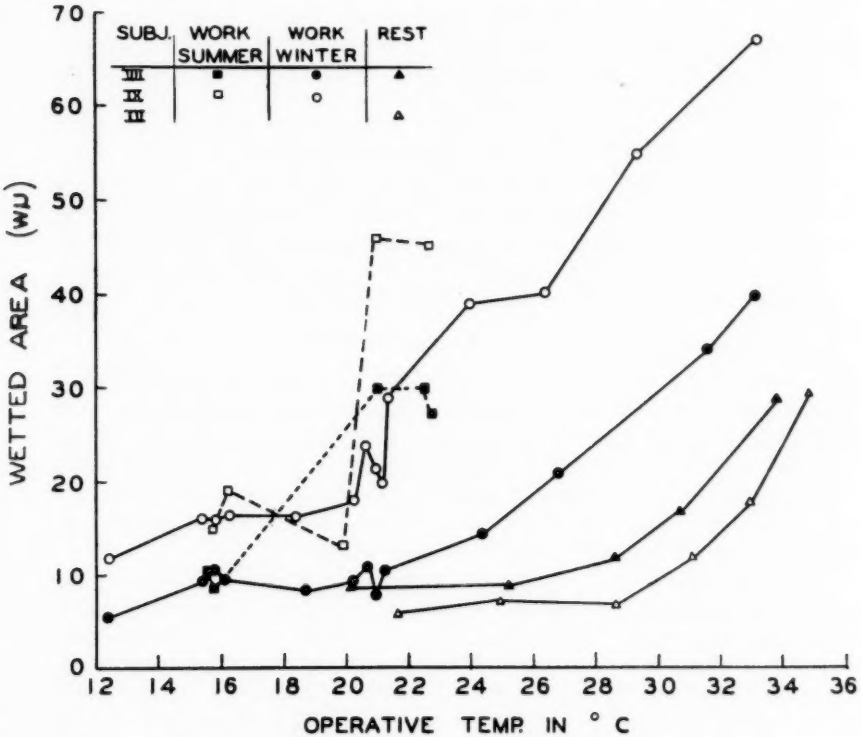

Fig. 6. Relation of wetted area to operative temperatures for resting subjects and for working subjects in summer and in winter.

standard atmospheric conditions (T_o, 21°C.) pedalling on the bicycle at a fixed rate (35 revolutions per minute) at five different loads, for which basic physiological data have been presented in table 5. We have arranged results for the two subjects for the following variations in work and metabolism.

	A	B	C	D	E
Work	8	19	45	57	70
Metabolism, mean	168	202	302	384	424

We present in figure 4 the mean values for four experiments at each of the three lower work rates and two experiments at each of the higher work rates, with respect to regional skin temperatures, rectal temperature, conductance and degree of wettedness.

It will be noted that the skin temperatures of head, upper extremities and lower extremities all rise slightly with increasing metabolic activity up to the fifth and highest metabolic rate, at which rate these temperatures fall slightly, apparently as a result of over-compensation due to very high evaporation. Trunk temperatures tend rather to fall with increased metabolic activity, very rapidly at the highest metabolic rate. This phenomenon is undoubtedly related to the fact that this is the area of the body where evaporative cooling is chiefly localized. Rectal temperature rises steadily with increasing metabolism. Conductance rises (except at the fifth work rate) and sweat secretion (w_μ) shows very great and progressive increase at metabolic activities above 200 kgm.-cal.

SUMMARY OF CONCLUSIONS

Study of the reactions of two unclothed male subjects pedalling on a stationary bicycle at such a rate as to increase their metabolism to over 300 kgm.-cal. per hour revealed the following differences, as compared to earlier results obtained with similar subjects at rest in a semi-reclining position (with a metabolism of 80–100 kgm.-cal.).

1. The radiation area is the same as that for the resting subject, approximately 70 per cent of the body surface area.

2. The convection constant for a subject sitting on the stationary bicycle but at rest is about 20 per cent less than that for the resting reclining subject, as a result of his more nearly vertical position. When the subject is pedalling at a rate of some 30 revolutions per minute, the convection constant is greatly increased; and this increase (due to the movements of the body and particularly of the legs) is equivalent in cooling power to an air movement of 30 to 40 cm. per sec. Under these conditions, the general body build of the subject has little influence on convection loss. Further increase in pedalling rate does not change the constant materially. Air movement produces its usual influence, but since this effect of air (or body) movement varies with the square root of velocity, the actual effect of even rather high air movements (superimposed on that of body movements) is relatively slight.

3. Increased sweat secretion, with consequent evaporative cooling, balances very perfectly the increased heat which must be dissipated as a result of high metabolism. The rate of sweat secretion increases three to five-fold for a two to three-fold rise in metabolism. Active sweat secretion sets in under conditions of winter adaptation at an operative temperature of 20° instead of at 30° with the resting subject. In summer the trigger

mechanism of sweat secretion is more sensitive and initiates active sweat secretion somewhere between 16° and 20°. At 21°C. T_o, the thermal reactions of the unclothed body are in balance with a metabolism of 350 kgm.-cal.

4. Evaporative regulation in the case of the working subject operates so effectively that skin temperature is held remarkably constant, actually being lower—at a given T_o—for the working than for the resting body. With increasing metabolism at a given T_o, the skin temperature of the trunk actually falls. Rectal temperature, however, rises appreciably under working conditions. It would seem, under these circumstances, clear that internal temperature—not skin temperature—must control the sweat-secreting mechanism.

5. A thermal sensation of pleasantness is produced in the unclothed working subject (with a metabolism of 300–400 kgm.-cal.) at a T_o of 16°, instead of 28° with the resting subject. In both instances, maximum comfort is experienced about 2° below the point where active sweat secretion begins.

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